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Aims and Scope:

The Journal of Physiological Sciences **publishes peer-reviewed original papers, reviews, short communications, technical notes, and letters to the editor, based on the principles and theories of modern physiology and addressed to the international scientific community. All fields of physiology are covered, encompassing molecular, cellular and systems physiology. The emphasis is on human and vertebrate physiology, but comparative papers are also considered. The process of obtaining results must be ethically sound.**

Fields covered:

- **Adaptation and environment**
- **Autonomic nervous function**
- **Biophysics**
- **Cell sensors and signaling**
- **Central nervous system and brain sciences**
- **Endocrinology and metabolism**
- **Excitable membranes and neural cell physiology**
- **Exercise physiology**
- **Gastrointestinal and kidney physiology**
- **Heart and circulatory physiology**
- **Molecular and cellular physiology**
- **Muscle physiology**
- **Physiome/systems biology**
- **Respiration physiology**
- **Senses**

Plenary Lectures
Academic Education Lectures
Named Lectures

Plenary Lecture 1

(March 21, 10:00～10:45, Room A)

PL1

Electron Tomography or the Challenge of Doing Structural Biology *in situ*

Baumeister, Wolfgang (*Max-Planck-Institute of Biochemistry, Germany*)

Electron cryotomography enables the structural analysis of non-repetitive pleomorphic structures, such as organelles or even whole cells providing unprecedented insights into their supramolecular organization. In conjunction with subtomogram classification and averaging molecular structures can be studied *in situ*, i.e. in their functional cellular environments. Recent developments such as the targeted micromachining of cells embedded in amorphous ice using correlative LM-EM techniques and focused ion beam technology open up new windows of opportunity for studying cellular ultrastructure. Studies of ribosomes and proteasomes *in situ* and of neurotoxic aggregates will illustrate the potential of this new approach to structural cell biology.

References:

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Plenary Lecture 2

(March 21, 10:45～11:30, Room A)

PL2

Structural physiology studied by cryo-electron microscopy

Fujiyoshi, Yoshinori (*Cellular and Structural Physiology Institute (CeSPI), and Graduate School of Pharmaceutical Sciences, Nagoya University, Japan*)

I am personally interested in molecular mechanisms, how education and experiences during human development influence the ability and personality of the adult. To challenge such a difficult question, structural and functional studies of membrane proteins are important, and thus I named this research field structural physiology. I would like to discuss mainly three topics of cell adhesive-channels. First, as an exceptional feature specific to AQP4 among 13 water channel isoforms, characteristic orthogonal arrays were observed and the array formation of AQP4 was regulated by the N-terminal palmitoylation of either Cys13 or Cys17, which was revealed by structure analysis of AQP4 2D-crystals [JMB 355, 628-39 (2006)] and subsequent freeze-fracture studies [BBA 1778, 1181-9 (2008)]. Large numbers of AQP4 molecules with cell adhesive-function are expressed in the glial lamellae of hypothalamus at which important brain functions such as thermo-, osmo- and glucose-sensory systems are thought to be carried out. For example, AQP4 might therefore be responsible for the pressure regulation in brain. The second topic is gap junction intercellular communication channels that allow a wide variety of solutes to pass through, and have critical roles in biologically important processes, such as, cardiac development, fertility, immune system and electrical signaling in the nervous system. The structures of connexin-26 were analyzed by electron crystallography [PNAS, 104 10034-9 (2007)] as well as X-ray crystallography [Nature 458, 597-602 (2009)], and we proposed plug gating model as a gating mechanism of the gap junction channel. As the third topic, we recently analyzed structure of claudin by X-ray crystallography and proposed a paracellular channel model [Science 344, 304-7 (2014)].

Plenary Lecture 3

(March 22, 10:30～11:15, Room A)

PL3

Receptors, Neurons, and Circuits: The Biology of Mammalian Taste

Zuker, Charles S.（*Howard Hughes Medical Institute and Columbia University, USA*）

The taste system is one of our fundamental senses, responsible for detecting and responding to sweet, bitter, umami, salty, and sour stimuli. In the tongue, the five basic tastes are mediated by separate classes of taste receptor cells each finely tuned to a single taste quality. In the cortex, each taste quality is represented in its own separate cortical field, revealing the existence of a gustotopic map in the brain. We study the logic of taste coding as a platform to understand how our brain creates an internal representation of the outside world and transforms sensory signals at the periphery into percepts, actions and complex behaviors.

Plenary Lecture 4

(March 22, 11:15～12:00, Room A)

PL4

Neural Map Formation in the Mouse Olfactory System

Sakano, Hitoshi（*School of Medicine, University of Fukui, Japan*）

In the mouse olfactory system, odorants are detected with ~1,000 different odorant receptor (OR) species expressed in the cilia of olfactory sensory neurons (OSNs). Each OSN in the olfactory epithelium (OE) expresses only one functional OR gene in a mutually exclusive and mono-allelic manner. Furthermore, OSNs expressing the same OR species converge their axons to a specific location in the olfactory bulb (OB) forming a glomerular structure. Because a given OR responds to multiple odorants and a given odorant activates multiple OR species, the odor information detected in the OE is topographically represented as the pattern of activated glomeruli in the OB[1].

A remarkable feature of axonal projection in the mouse olfactory system is that ORs play an instructive role in projecting OSN axons to the OB. For dorsal-ventral (D-V) projection, anatomical location of OSN cells within the OE regulates both OR gene choice and expression levels of axon guidance molecules, thus indirectly correlating the OR identity to the glomerular location along the D-V axis[2]. In contrast, anterior-posterior (A-P) projection is totally independent of the positional information of OSN cells, but instead dependent on the expressed OR species[3]. We have recently found that A-P targeting is regulated by the agonist-independent baseline activity of ORs using cAMP as a second messenger[4]. OR-derived cAMP signals also regulate the expression of glomerular segregation molecules for the map refinement through local sorting of OSN axons[5]. Unlike A-P projection molecules, glomerular segregation molecules are regulated by stimulus-driven neuronal activity[4].

Here, we discuss the recent progress in the neural map and circuit formation in the mouse olfactory system.

References

1) *Mori, K. and Sakano, H.*: Ann. Rev. Neurosci. 34, 465 (2011).
2) *Takeuchi, H., et al.*: Cell 141, 1056 (2010).
3) *Imai, T., et al.*: Science 325, 585 (2009).
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5) *Serizawa, S., et al.*: Cell 127, 1057 (2006).

Plenary Lecture 5

(March 23, 10:30～11:30, Room A)

PL5

Molecular Dissection of Autophagosome Formation in Yeast

Ohsumi, Yoshinori (*Integrated Research Institute, Tokyo Institute of Technology, Japan*)

Autophagy is well a conserved degradation process of cytoplasmic constituents in the lysosome/vacuole. Recently it is getting clear that autophagy plays important roles in so many physiological events and is related to diseases. More than 26 years ago we first found autophagy in yeast induced by nutrient starvations by light microscopic observation. Taking advantage of the yeast system, we started genetic approach to dissect the process, and successfully isolated many autophagy-defective mutants. Subsequent identification of ATG genes revealed unique set of genes involved in membrane dynamics during autophagy. These genes were mostly conserved in mammals and plants and most other eukaryotes. These findings triggered a vast of autophagy research in various organisms. We know now that 18 *ATG* genes are essential for starvation-induced autophagy in yeast. They consist of six functional units, namely the Atg1 protein kinase and its regulators, the PI3 kinase complex, the Atg2-Atg18 complex, the membrane protein Atg9, and two unique ubiquitin-like conjugation systems. Then we have been focusing to elucidate the structure and function of each Atg protein. Atg proteins function concertedly in membrane dynamics during the formation of autophagosome. Recent studies on the Atg proteins, especially early steps of the PAS assembly will be presented. In addition recent physiological roles of autophagy in yeast will be discussed.

Academic Education Lecture 1

(March 21, 14:00～14:45, Room A)

EL1

Kinesin Superfamily Molecular Motors, KIFs: Intracellular Transport, Regulation of Higher Brain Function, and Development and Diseases

Hirokawa, Nobutaka (*Graduate School of Medicine, The University of Tokyo, Japan*)

Academic Education Lecture 2

(March 21, 14:45～15:30, Room A)

EL2

Integrative research on bio-system bridging from single molecules to organ

Yanagida, Toshio (*Graduate School of Frontier Biosciences, Osaka University, Japan*)

S. Hagiwara Memorial Lecture

(March 23, 13:30～14:15, Room A)

NL1

The role of cortical areas MT/MST in short-latency ocular tracking

Kawano, Kenji (*Graduate School of Medicine, Kyoto University, Japan*)

Whenever we move around in the environment, the observer's movements activate the vestibular organs and are then compensated by the vestibulo-ocular reflexes (VORs). However, the VORs are not always perfect and the visual acuity is severely impaired if the images of interest on the retina move excessively. Recent studies revealed three distinct visual tracking eye movements with ultra-short latencies (~60 ms in monkeys), which are thought to help reduce the residual visual disturbances. One of these eye movements is 'ocular following', which deals with the visual stabilization problems confronting the observer who looks off to one side. Two other eye movements, 'disparity vergence' and 'radial-flow vergence', deal with the binocular fusion problems of the observer who looks in the direction of heading.

To understand the neural mediation of these tracking eye movements, we focused on the role of the middle temporal (MT) and medial superior temporal (MST) areas within the superior temporal sulcus (STS) of the monkey's cortex, since these areas are known to contain many neurons that respond vigorously to visual motion with directional selectivity and others that are sensitive to binocular disparity or to the patterns of optic flow experienced by the moving observer. We recorded single unit activities and made focal chemical lesions in these areas in monkeys. The results were consistent with the hypothesis that the MT/MST areas are primary sites for initiating all three visual tracking eye movements at ultra-short latencies.

S. Tawara Memorial Lecture

(March 23, 14:15～15:00, Room A)

NL2

Intracellular Ca^{2+} in striated muscle: measurement and physiological significance

Kurihara, Satoshi (*The Jikei University School of Medicine, Japan*)

Intracellular Ca ion (Ca^{2+}) plays a pivotal role in muscle contraction. In the present Tawara Memorial Lecture, I will present the intracellular Ca^{2+} concentration change measured with the Ca^{2+} sensitive photoprotein aequorin in mammalian cardiac muscles, and will discuss the molecular mechanism of the length-dependent change of tension in cardiac muscle (the Frank-Starling law of the heart). If the papillary muscle of the rat or ferret was stretched from a shorter length to the length to produce maximal tension (Lmax), tension was increased without a change in the peak Ca^{2+} signal (Ca^{2+} transient, CaT). However, the relaxation time was prolonged and the decay time of CaT was shortened. If muscle length was quickly shortened from Lmax to a shorter length during a twitch contraction, tension was promptly decreased and then re-developed. In response to quick release, the CaT showed a transient increase (hump) in the falling phase. The magnitude of the hump was correlated with the magnitude of tension reduction rather than with muscle length. If the preparation was treated with 2,3-butanedione monoxime, tension disappeared, but the CaT was not greatly affected. In the 2,3-butanedione monoxime-treated preparation, quick release did not induce a hump in the CaT. Thus, the change in muscle length affects the Ca^{2+} affinity of the Ca^{2+}-binding protein troponin through cross-bridge attachment and detachment. The measurement of the intracellular Ca^{2+} concentration is essential for understanding the molecular mechanism of cardiac muscle contraction.

President's Symposium

President's Symposium 1

Brain and hormones: Their seamless interaction between structure and function from molecular to behavioural level

(March 21, 8:30～10:00, Room A)

PS1-1

Structural studies in neuroendocrine research. Lessons from the past; clues for the future

Morris, John (*Department of Physiology, Anatomy & Genetics, University of Oxford, Oxford, UK)*

Structural studies have played a major role in the understanding of the functions of neuroendocrine systems. As new imaging techniques have been devised it has proved possible to look in greater and greater detail into the structure of neuroendocrine tissues, probing down to the level of individual molecules. While these studies have become increasingly good at providing an answer to "what" and "where" questions, in experiments that use an anatomical end-point it has only been when careful manipulation has been applied to the tissues that real advances in understanding function have been made. Most structural techniques provide only static images - literally single frames in the movie of living tissues. Furthermore, living, behaving organisms comprise many different tissue systems and our scientific exploration is only gradually getting to grips with ways in which the different systems and tissues interact to organise complex behaviours. In generating simple systems to apply Ockham's razor experimentally, we inevitably lose sight of the relative quantitative effect of all the competing signals involved.This presentation will review and analyse the ways in which investigations of the anatomy of neuroendocrine tissues has contributed to some of the milestones of increased understanding of the functional behaviour of neuroendocrine systems and entire organisms. It will also try to peer into the rather opaque crystal ball of the future by drawing lessons from past successes.

(COI: No)

PS1-2

Challenge to visualize/regulate physiological functions of neurohypophysial hormones

Ueta, Yoichi (*Dept. Physiol. Sch. Med. Univ. Occup. and Environ. Health, Kitakyushu, Japan)*

Neurohypophysial hormones, arginine vasopressin (AVP) and oxytocin (OXT) are synthesized in the magnocellular neurosecretory cells (MNCs) localized in the hypothalamic paraventricular (PVN) and the supraoptic nuclei (SON) that project their axon terminals into the posterior pituitary (PP). Recent studies have revealed that AVP and OXT are secreted not only into the systemic circulation from the axon terminals in the PP but also in the central nervous system from the somatodendrites of the MNCs. Nowadays, central actions of AVP and OXT are known such as social behavior, pair-bonding and maternal behavior. We challenged to visualize MNCs that synthesize AVP and OXT with their neuronal activities (the c-fos gene expression) simultaneously in transgenic rats that express the AVP-eGFP (or OXT-mRFP1) fusion gene and the c-fos-eGFP (or mRFP1) fusion gene. Recently, we have also challenged to regulate the neuronal activities of MNCs by a light-activated ion channel in transgenic rats that express the AVP-eGFP and channelrhodopsin 2 fusion gene. These genetic modified animals enable us to visualize/regulate neurohypophysial hormone dynamics in in vitro and in vivo preparation from vesicle, neuronal activity to behavior.

(COI: No)

PS1-3

Vasopressin: from synthesis to secretion

Leng, Gareth; Macgregor, Duncan (*Centre for Integrative Physiology, University of Edinburgh, Edinburgh, UK)*

In the forty years since the first electrophysiological recordings from identified vasopressin neurons, our understanding of these cells has come a long way – but how well do we really understand them? One test of our understanding is whether we can express it in a computational model that can reproduce the complex behaviour of vasopressin cells in a manner that is quantitatively precise, but which also can yield novel predictive insights. We have developed a computational model of the vasopressin cell that accounts for its characteristic electrophysiological behaviour (the distinctive phasic patterning of spike activity in individual cells), and, by introducing variability in key parameters we can generate a population of model cells that closely mimics the range of electrophysiological phenotypes observed *in vivo* (MacGregor & Leng, PLoS Comput Biol. 2012; 8(10): e1002740). We extended that spiking model to include a representation of stimulus-secretion coupling derived from experimental data, building a model population that displays the dynamics of secretion from the population as well as from individual cells (Macgregor & Leng, PLoS Comput Biol. 2013; 9(8): e1003187). Now we have extended that model further, to incorporate synthesis of vasopressin and its activity dependent regulation. This enables us to simulate the behaviour of the vasopressin system in response to diverse challenges – including challenges that result in depletion of the pituitary stores of vasopressin. Finally, we are extending the model to simulate dendro-dendritic interactions between vasopressin cells, to better understand how this intercommunication impacts on the physiological behaviour of the vasopressin system.

(COI: No)

PS1-4

Sex steroid feedback and brain programming -a role of kisspeptin neurons-

Tsukamura, Hiroko (*Grad. Sch. Bioagricultural Sci., Nagoya Univ., Nagoya, Japan)*

Mammalian reproductive function is regulated by the hypothalamus-pituitary-gonadal axis. The mechanism is sexually differentiated and the differentiation is considered to be due to the brain programming by perinatal sex steroids. Kisspeptin neurons, which play a critical role in reproduction via controlling gonadotropin-releasing hormone (GnRH)/gonadotropin release, show sexual dimorphism in the rodent brain: many kisspeptin neurons in female anteroventral periventricular nucleus (AVPV) while few in males. The present paper first focuses on the organizational effect of neonatal steroids causing the sexual differentiation of kisspeptin neurons and consequent GnRH/luteinizing hormone (LH) surge generating mechanism. We found that neonatal steroids decrease AVPV kisspeptin expressions, resulting in the failure of GnRH/LH surge in male rats. The neonatal steroids failed to affect kisspeptin neurons in the arcuate nucleus (ARC), which is considered to regulate GnRH/LH pulses. Second, the paper focuses on the functional effect of estrogen in adulthood, which is responsible for estrogen positive and negative feedback effects on GnRH/LH release to induce GnRH/surge and suppress GnRH/LH pulses, respectively. More specifically, the epigenetic mechanism involved in the estrogen feedback on kisspeptin expression in adult rodent brain will be discussed. This work was supported in part by the Research Program on Innovative Technologies for Animal Breeding, Reproduction (REP2002), and Vaccine Development and the Science and technology research promotion program for agriculture, forestry, fisheries and food industry.

(COI: No)

PS1-5

An integrated system of environmental signals on the reproductive neuroendocrine axis

Ozawa, Hitoshi (*Dept. Anat. Neurobiol., Grad.Sch. Med., Nippon Med. Sch., Tokyo, Japan)*

Reproductive neuroendocrine regulations are mainly originated and/or integrated at HPG axis, arising from 1) the hypothalamus, where a group of scattered neurons secretes gonadotropin-releasing hormone (GnRH) for stimulating gonadotropins from the gonadotroph in the anterior pituitary; 2) the anterior pituitary, where gonadotrophs secrete the gonadotropins for promoting the gonadal maturation, and 3) the gonados, which secrete sex steroids. In addition, feedback loops also regulate within this axis for facilitating the homeostatic regulation of reproductive neuroendocrine system in different physiological conditions. Recent advance in our understanding of the controlling GnRH, therefore the functions of gonados came with discover of the kisspeptin and their receptor, GRP54. Kisspeptin neurons are observed in the anteroventral periventral (AVPV) and the arcuate (Arc) nuclei in the rat brain. It is reported that kisspepitn neurons express sex steroid receptors, and the leptin receptor and cortcotropin-releasing hormone receptor. This means that kisspeptin neurons directory receive the negative feedback signal of HPG axis, the energy (nutritional) information, and the stress response signal, then the kisspeptin neurons relay these environmental information to the HPG axis. So, the kisspeptin neurons may be possible to understand integrating neurons of the different physiological information to facilitate the homeostatic regulation of the HPG axis. In the paper, I would like to introduce a new concept of kisspeptin-HPG axis, which is interacted with the energy regulation and the stress response.

(COI: No)

President's Symposium 2

Structure and function of biological membranes: viewed from molecules and their nano-environments

(March 23, 9:00～10:30, Room A)

PS2-1

Crystal Structure of Voltage Sensor Domain Protein

Nakagawa, Atsushi[1,2] ([1]*Inst for Protein Res, Osaka Univ;* [2]*CREST, JST)*

X-ray crystallography is a powerful technique to determine the three-dimensional atomic structures of biological macromolecules, such as proteins. The atomic structure gives valuable information to understand the function of the biological macromolecules.

The voltage-gated ion channels are the members of voltage-sensing protein family, which is regulated by membrane potential changes. The voltage-gated ion channel is consisted from two distinct domains, called a voltage sensor domain (VSD) and a channel domain. When the sensor domain senses membrane potential changes, the S4 helix in the sensor domain changes its orientation and transmits the signal to the channel domain, and open and close the gate that is formed by four domains of the tetramer of the molecules. Recently new protein family, which has VSD but lacks channel domain, has been identified and is named voltage sensor domain protein. Voltage-gated proton channel, named Hv1 or VSOP, is a member of this family. The VSD of Hv1 plays dual roles of voltage sensing and proton permeation. It is required for high-level superoxide production by phagocytes through its tight functional coupling with NADPH oxidase to eliminate pathogens. Hv1 is also expressed in human sperm and has been suggested to regulate motility through activating pH-sensitive calcium channels. The activities of Hv1 also have pathological implications, such as exacerbation of ischemic brain damage and progression of cancer.

The crystal structure of mouse Hv1 (mHv1) in the resting-state was determined at 3.45A resolution, and it provides a novel platform for understanding the general principles of voltage sensing and proton permeation.

(COI: No)

PS2–2

Physiological functions revealed by looking at nanoscale distribution of membrane lipids

Fujimoto, Toyoshi (*Grad. Sch. Med. Nagoya Univ., Nagoya, Japan*)

The biological membrane is a highly dynamic structure, but most physiological reactions occur in restricted areas. Therefore, to study phenomena in the membrane, e.g., signal transduction, it is important to know how membrane molecules, both proteins and lipids, are located in the smallest-possible scale. Electron microscopy is a powerful tool for this purpose, but conventional techniques are not satisfactory because those used for proteins are not applicable to or not appropriate for lipids. One major problem for lipids is that they do not react with aldehydes and remain mobile even after fixation.

To circumvent this "unfixability" problem, we have been working on an EM method utilizing physical fixation. In this method, cells are quick-frozen to stop molecular motion instantaneously; then a membrane is split into two leaflets by freeze-fracturing, after which membrane molecules are immobilized by vacuum evaporation of platinum and carbon; to this physically-stabilized membrane preparation, specific probes are applied to label target molecules.

By using this method, we can observe not only the two dimensional distribution of membrane lipids (and proteins as well) but also the asymmetry that lipids show between the outer and inner leaflets. Because membranes are retained in a stable form in the freeze-fracture replica, they can be subjected to various chemical treatments that may perturb membrane structures when applied to native membranes. I would like to show the results on several different lipids, including gangliosides and phosphoinositides [PI(4, 5)P_2, PI(3)P, PI(3, 5)P_2] and discuss their physiological implications.

(COI: No)

PS2–3

The class III PI3K and phosphatidylinositol 3-phosphate ensure structural and functional integrity of cardiomyocytes

Sasaki, Takehiko[1,2]; Kimura, Hirotaka[1,2]; Eguchi, Satoshi[1]; Sasaki, Junko[1]; Mizushima, Noboru[3] ([1]*Dept Med Biol, Grad Sch Med, Akita Univ, Akita, Japan;* [2]*Res Center for Biosignal, Akita Univ, Akita, Japan;* [3]*Dept Biochem and Mol Biol, Grad Sch Med, Tokyo Univ, Tokyo, Japan*)

Vps34, the catalytic subunit of the class III phosphoinositide 3-kinase complex, phosphorylates phosphatidylinositol to produce phosphatidylinositol-3- phosphate (PtdIns3P). The principal role of PtdIns3P is to recruit cellular proteins to specific membrane domains where they function. To date, more than 100 proteins with a range of functions have been reported to bind to PtdIns3P. Thus, PtdIns3P synthesis by Vps34 is considered important for a variety of cellular activities.Here, we report that Vps34 is involved in the etiology of certain cases of idiopathic cardiomyopathy. Vps34 expression in the heart was markedly reduced in a subset of patients with hypertrophic cardiomyopathy. In accordance with the observation, muscle-specific deletion of *Pik3c3* encoding Vps34 in mice led to thickening of the left ventricular wall, reduced cardiac contractility, bundle branch block and arrhythmia. All these mutants died suddenly between postnatal day 80 (P80) and P110, suggesting a protective role for Vps34 against the occurrence and progression of heart failure. We will present the molecular mechanisms behind these abnormalities and discuss the interplay between autophagy and ESCRT machinery, the two protein degradation systems diverge from PtdIns3P, in the context of myofibril organization.

(COI: No)

PS2–4

Specialized membrane nanodomain organized by lipid modification ~ Synaptic organization regulated by PSD-95 palmitoylation machinery ~

Fukata, Masaki[1,2]; Sekiya, Atsushi[1,2]; Murakami, Tatsuro[1,2]; Yokoi, Norihiko[1,2]; Kobayashi, Kenta[1,3]; Fukata, Yuko[1,2] ([1]*Div Membrane Physiol, NIPS, Japan;* [2]*Dept Physiol, SOKENDAI, Japan;* [3]*Viral Vector, NIPS, Japan*)

Precise regulation of protein assembly at specialized membrane domains is essential for diverse cellular functions including synaptic transmission. However, it incompletely understood how protein clustering at the plasma membrane is initiated, maintained and controlled. Protein palmitoylation, a common reversible lipidation, regulates protein targeting to the plasma membrane. Such modified proteins are enriched in these specialized membrane domains. Recently, we found that endogenous palmitoylated postsynaptic density protein 95 (PSD-95), a representative postsynaptic scaffold, is partitioned into multiple discrete subdomains (nanodomains) in a dendritic spine in cultured hippocampal neurons. PSD-95 in nanodomains undergoes continuous de/repalmitoylation cycles driven by local palmitoylating activity, ensuring the maintenance of compartmentalized PSD-95 clusters within individual spines. Acutely induced plasma membrane insertion of DHHC2 palmitoylating enzyme triggers specific accumulation of PSD-95 at the plasma membrane, and this plasma membrane-inserted DHHC2 is essential for postsynaptic nanodomain formation. Furthermore, we obtained the candidate for the membrane-bound enzyme to depalmitoylate PSD-95 and disperse synaptic PSD-95 clusters. We propose that synaptic palmitoylation machinery defines subsynaptic nanodomains through constituting local palmitoyl cycles on PSD-95 and determines the geometry of postsynaptic densities.

(COI: No)

Meeting Symposium

Meeting Symposium 1

Body in the world - coordinates in the brain-

(March 21, 8:30～10:00, Room C)

MS01-1

Transformation of visual motion from retinotopic to spatiotopic coordinates in the cortical areas MT and MST

Inaba, Naoko[1,2] ([1]*Research and Educational Unit of LIMS, C-PIER, Kyoto University, Kyoto, Japan;* [2]*Dept Integrative Brain Sci, Grad Sch Med, Kyoto University, Kyoto, Japan*)

The retinal image of a visual scene is constantly moving due to our eye movements, yet the visual world around us appears to remain stationary. To understand the neural mechanisms for this perceptual stability, we studied the activities of neurons in two cortical regions, medial superior temporal (MST) area, and its major input, middle temporal (MT) area in awake behaving monkeys. We measured neuronal responses to a large-field textured background that moved briefly at various speeds and directions during smooth pursuit and stationary fixation. The speed-tuning and direction-tuning of neurons were studied to determine if the speed preference and the direction selectivity of each neuron remained the same even during pursuit. We found that most MST neurons were more sensitive to the stimulus motion in space irrespective of the speed and direction of pursuit; *i.e., in spatiotopic coordinates*. On the other hand, most MT neurons selectively responded to the image motion on the retina; *i.e., in retinotopic coordinates*. The result suggests that the MST neurons compensate, at least in part, for retinal image motion resulting from pursuit eye movements. Since the MST area receives visual inputs from the MT area and the extra-retinal information during pursuit is known to be in the MST area, transformation of visual motion from retinotopic to spatiotopic coordinates is likely achieved by neuronal processing between the cortical areas MT and MST.

(COI: No)

MS01-2

Equations of motion for reaching dynamics and coordinate systems in frontoparietal motor system

Tanaka, Hirokazu (*School of Information Science, JAIST*)

How neurons in the primary motor cortex (M1) control arm movements and how a visual input is transformed into motor control signals in the frontoparietal motor network are not yet understood. Here I show that the equations of motion governing reaching simplify when expressed in spatial coordinates, known as Newton-Euler dynamics in robotics. In this fixed reference frame, joint torques are the sums of vector cross products between the spatial positions of limb segments and their spatial accelerations and velocities. The consequences that follow from this model explain many properties of neurons in M1, including directional broad, cosine-like tuning, non-uniformly distributed preferred directions dependent on the workspace, and the rotation of the population vector during arm movements. Remarkably, the torques can be directly computed as a linearly weighted sum of responses from cortical motoneurons, and the muscle tensions can be obtained as rectified linear sums of the joint torques. The model is further extended to the frontoparietal network; the parietal reach area represents a goal-directed hand movement in the retinal coordinates, which in turn is transformed into body-centered coordinates in the premotor areas. The superior parietal lobule including area 5d operates as a forward model computing a prediction of movement from efference copies from the premotor areas. In summary, reaching dynamics expressed in spatial coordinates provides a unifying framework for understanding the functional roles and coordinate systems used in the frontoparietal motor network.

(COI: No)

MS01-3

Representing a target in terms of the background: functions of the background coordinate and its neural correlates

Kitazawa, Shigeru[1,2,3] ([1]*Dept Brain Physiol, Grad Sch Med, Osaka Univ, Osaka, Japan;* [2]*Dynamic Brain Netw Lab, Grad Sch Frontier Biosci, Osaka Univ, Osaka, Japan;* [3]*CiNet, NICT, Osaka Univ, Osaka, Japan*)

Our brain represents a target position in terms of our body parts, like in retinal or craniotopic coordinates (egocentric coordinates). In addition, many previous studies reported that a target position can be represented in terms of landmarks in the background (allocentric coordinate). However, it was generally believed that it takes time to represent a target in terms of landmarks, and that the importance of the allocentric representation increases in such un-ecological situations when we are forced to memorize a target position for some period (~5 s). We have recently shown that a target can be represented immediately (within 300 ms) in terms of a frame in the background, and that the background coordinate is utilized to dissociate the effect of target motion from the error resulting from the issued motor control signals. We have further shown by using an fMRI-adaptation technique that neural correlates of the background coordinate is distinct from those of the retinal and craniotopic coordinates, and reside in several regions in the right hemisphere, including the precuneus, the middle occipital gyrus, and the middle temporal gyrus. We finally discuss another possible function of the background coordinate in stabilizing our visual experience.

(COI: No)

MS01-4

Representations of spatial and non-spatial information in the hippocampus

Fujisawa, Shigeyoshi (*RIKEN BSI, Wako, Japan*)

Hippocampal pyramidal cells display place-selective activities in the environmental space, and traveling in the space arises sequential activities of the place cells. Importantly, sequential activities of hippocampal cells are also observed in the representations of information dissociated from 'space', such as the representation of 'time'. We performed large-scale extracellular single-unit recordings from the hippocampal CA1 in rats performing a working memory task, and also found sequentially organized neuronal activities which differentiated between different odor cues during working memory periods. Here we discuss about the roles of hippocampal cell assembly sequences on coordinates of space and time in the brain.

(COI: No)

Meeting Symposium 2

Exercise physiology in advanced aging society: basic and applied aspects

(March 21, 8:30～10:00, Room E)

MS02-1

The stromal cells interaction and formed 3D network following acute muscle trauma

Kobayashi, Masatoshi[1]; Ohta, Keisuke[2]; Nakamura, Kei-ichiro[2]; Sakurai, Tadayoshi[1]
([1]*Nippon Sports Sci. Univ., Tokyo, Japan;* [2]*Kurume Univ., Fukuoka, Japan*)

It has been reported that the mononuclear cells which locate in the interstitial space of damaged muscle tissue might take part in muscle fibers repair. However, the morophology and the strain of these cells are not clear.
In this study, trauma loaded rat skeletal muscles (gastrocnemius) were observed with focused ion beam scanning electron microscope (FIB/SEM) into 600 slice pictures, which were reconstructed into three dimensional images by the program to develop the localization and formation of stromal cells.
As a result, we observed that many cells invaded it in muscle fibers, and three kinds of stromal cells were identified in the interstitial space of the gastrocnemius muscles at the 1st and the 2nd day after muscle damage. The first appeared cells had spindle shaped form. The second cells had many rough endoplasmic reticula (r-ER). The third cells were similar to granulocyte. These 3 types of cells were contacted one another and formed network.
It was suggested that these stromal cells may exchange some information which plays some important roles in regeneration process after muscle damage. These "cell to cell contact" might have an important biomedical meaning in "the transformation of cells", "the cell proliferation" and "the invasion to muscle fibers".

(COI: No)

MS02-2

Exercise-induced brain glycogen decrease and supercompensation

Matsui, Takashi[1,2]; Kawanaka, Kentaro[2]; Soya, Hideaki[3]
([1]*JSPS Research Fellow-SPD;* [2]*Dept Health and Nutrition, Niigata Univ of Health and Welfare, Niigata, Japan;* [3]*Lab Exerc Biochem, Univ of Tsukuba, Ibaraki, Japan*)

Exercise activates not only skeletal muscles but also brain neurons. Although the brain is fuelled by carbohydrates, how brain carbohydrate metabolism functions and adapts to exercise remains uncertain. Muscle glycogen is broken down and decreases during exercise and is replenished to above basal level with rest after exercise, a process called supercompensation (Nature, 1966). Muscle glycogen supercompensation is an important phenomenon because it is the basis of exercise adaptation in muscle carbohydrate metabolism (the increase in muscle glycogen storage). Recently, lactate borne from brain glycogen stored in astrocytes has been shown as a critical energy source to retain neuronal functions. We here found, for the first time, that prolonged exhaustive exercise induces the decrease and supercompensation in glycogen in several brain loci such as the cortex and hippocampus, as like in muscles. We also confirmed that four weeks of chronic exercise that elevates endurance and cognitive capacity increases the glycogen storage in the cortex and hippocampus. Our results demonstrated the exercise adaptation of brain carbohydrate metabolism in the cortex and hippocampus similar to muscles, which is likely due to accumulative effects of the brain glycogen supercompensation after it decreases with exercise. The exercise adaptation of brain carbohydrate metabolism in the cortex and hippocampus, which control physical and cognitive functions, could contribute to exercise-improved endurance and cognitive capacities.

(COI: No)

MS02-3

Dietary supplementation with ubiquinol-10 decelerates senescence and age-related hearing loss in SAMP1 mice via the activations of sirtuins and their downstream molecules

Sawashita, Jinko[1,2]; Tian, Geng[2]; Higuchi, Keiichi[1,2]
([1]*Dept Biol Sci Intractable Neurol Dis, IBS-ICCER, Shinshu Univ, Matsumoto, Japan;* [2]*Dept Aging Biol, Inst Pathogenesis Preventive Med, Shinshu Univ Graduate Sch Med, Matsumoto, Japan*)

We have revealed previously that supplementation with reduced form of coenzyme Q_{10} (ubiquinol-10, QH_2) significantly delayed senescence in Senescence-Accelerated Mouse Prone-1 (SAMP1) mice (1, 2). In our recent study, we found that QH_2 can also delay progression of age-related hearing loss in SAMP1 mice (3). Here, we report that dietary QH_2 supplementation prevents age-related decreases in the expression of sirtuins, which results in the activation of PGC-1 α , a major factor that controls mitochondrial biogenesis and respiration, as well as SOD2 and IDH2, which are mitochondrial antioxidant enzymes. QH_2 supplementation also increases mitochondrial complex I activity and numbers of mitochondria, and decreases levels of oxidative markers. Furthermore, QH_2 increases cAMP levels by activating adenylate cyclase and repressing phosphodiesterase that, in turn, activated CREB and AMPK in HepG2 cells. These results suggest that QH_2 supplementation may enhance mitochondrial activity by increasing levels of sirtuins and their downstream molecules, and is followed by protection against the progression of aging and symptoms of age-related diseases (3).
1, Yan J *et al.* (2006) Exp Gerontol 41:130-140
2, Schmelzer C *et al.* (2010) Mol Nutr Food Res 54:805-815
3, Tian G *et al.* (2014) Antioxid Redox Signal 20:2606-2620

(COI: No)

MS02-4

Effects of dairy products intake on thigh muscle strength and *NFκB2* gene methylation during walking training in middle-aged and older women

Masuki, Shizue[1,3]; Taniguchi, Shun-ichiro[2,3]; Nose, Hiroshi[1,3]
([1]*Sports Med Sci;* [2]*Mol Oncol, Shinshu Univ Grad Sch Med, Matsumoto, Japan;* [3]*IBS, Shinshu Univ, Matsumoto, Japan*)

Muscle atrophy with aging is the fundamental causes for lifestyle-related diseases. As for the mechanisms, muscle atrophy may induce release of cytokines from the muscle or other organs, causing chronic systemic inflammation. In this study, we assessed whether post-exercise dairy products intake (PEDPI) during 5-month interval walking training (IWT) enhanced the increase in thigh muscle strength and ameliorated the susceptibility to inflammation in middle-aged and older women. Subjects (n=37, 54-74 yr) were randomly divided into 3 groups: IWT alone (CNT, n=12), IWT + PEDPI of low dose (LD, n=12; 4g protein, 3g carbohydrate, and 3g fat) or 3 times higher dose (HD, n=13). They were instructed to repeat $\geq$5 sets of fast and slow walking for 3 min each at $\geq$70% and 40% peak aerobic capacity for walking, respectively, per day $\geq$4 days/wk. We determined thigh muscle strength and promoter methylation in *NFκB2* gene, a well-known transcriptional regulator of inflammation, by PyroSequencing before and after IWT. After IWT, thigh muscle strength significantly increased in HD ($P<0.05$) while not in CNT or LD despite similar training achievement among groups ($P>0.5$). Moreover, an increase in *NFκB2* methylation after IWT was 43±9% in HD, greater than 9±9% in LD ($P<0.05$) and -10±9% in CNT ($P<0.001$), suggesting greater suppression of pro-inflammatory cytokines in HD. Thus, PEDPI enhanced the increases in thigh muscle strength and *NFκB2* methylation by IWT in middle-aged and older women.

(COI: No)

Meeting Symposium 3

Neuronal Specializations of Auditory Temporal Coding

(March 21, 14:00～15:30, Room G)

MS03-1

Synaptic plasticity interacts with postsynaptic membrane kinetics in the chick cochlear nucleus

Burger, Michael R.; Oline, Stefan (*Lehigh University, Bethlehem, PA, USA*)

Auditory stimuli are processed in parallel, frequency-tuned circuits. Auditory nerve fibers (nVIII) impart frequency tuning onto their postsynaptic targets in nucleus magnocellularis (NM). NM neurons express specializations that reflect the characteristic frequency (CF) of their nVIII inputs, and may confer computational specificity. Our previous work demonstrated a gradient of synaptic input properties according to CF where short term depression was strongest for neurons processing low frequencies. We are now evaluating how the postsynaptic neurons integrate inputs in depressed and rested conditions. An efficient way to evaluate postsynaptic properties is by examining responses to injected ramp or frequency modulated Zap-currents. Low CF neurons exhibited longer integration times in response to ramp currents, with spikes following less steep and more prolonged stimuli compared with high CF cells. Responses to ZAP currents were almost all low pass for low CF neurons, while nearly a third of high CF neurons were band-pass. These responses were strikingly adaptive to input conditions. Depolarization shifted frequency selectivity of high CF cells toward higher peak response frequency (f0), enhancing the band pass output of these neurons. Together, these data suggest that computational strategy for spike initiation in NM may depend on both input conditions and frequency selectivity. Interestingly, the ability for high CF neurons to alternate between low- and band-pass filtering may indicate a stimulus-dependent switch, between relay and integrate-and-fire strategies for neural computation.

(COI: No)

MS03-2

Roles of phasic inhibition in coincidence detector neurons of birds

Yamada, Rei (*Dept Cell Physiol, Grad Sch Med, Nagoya Univ, Nagoya, Japan*)

The interaural time difference (ITD) is crucial in localizing the sound source particularly for low-frequency sounds. In birds, neurons in nucleus laminaris (NL) detect the coincidence of bilateral excitatory inputs and change their firing rate as a function of the ITD. Inhibitory synapses are proposed to be significant for accurate ITD detection and tonic inhibition is known to control the gain of coincidence detection, which enhances the ITD sensitivity of NL neurons for strong-intensity sound. In this symposium, I will introduce a phasic inhibition that was found in chicken brain slices, which may further enhance ITD sensitivity for low-frequency sound. During whole-cell recording from low-frequency NL neurons, stimulation to the ipsilateral projection fibers generated a polysynaptic IPSC that follows EPSC with 1-2 ms latency. In addition, GABA-positive neurons are distributed near the NL and generated the IPSCs in NL neurons when photoactivated by a caged glutamate. These results suggest that these GABAergic neurons are interneurons that mediate phasic inhibition to the NL neurons. Model simulations demonstrated that these phasic IPSCs narrow the time window for coincidence detection and increase the contrast of ITD-tuning particularly when the excitatory inputs are weak. Furthermore, cooperation of the phasic and tonic inhibitions effectively increases the contrast of ITD-tuning over a wide range of excitatory input levels. We propose that the complementary interaction between phasic and tonic inhibitions is the neural mechanism that improves ITD sensitivity for low-frequency sound in the NL.

(COI: No)

MS03-3

Inhibition Tunes Coincidence Detection in the Auditory Brainstem

Myoga, Michael Hideki[1,2] ([1]*Max-Planck-Inst. für Neurobiologie, Germany;* [2]*Ludwig-Maximilians-University Munchen, Grosshaderner Str.2, Planegg-Martinsried, Germany*)

Neurons in the medial superior olive (MSO) detect microsecond differences in the arrival time of sounds between the ears (interaural time differences or ITDs), a crucial binaural cue for sound localization. Synaptic inhibition has been implicated in tuning ITD sensitivity, but the cellular mechanisms underlying its influence on coincidence detection are debated. Here we determine the impact of inhibition on coincidence detection in adult Mongolian gerbil MSO brain slices by testing precise temporal integration of measured synaptic responses using conductance-clamp. We find that inhibition dynamically shifts the peak timing of excitation, depending on its relative arrival time, which in turn modulates the timing of best coincidence detection. Inhibitory control of coincidence detection timing is consistent with the diversity of ITD functions observed in vivo and is robust under physiologically-relevant conditions. Our results provide strong evidence that temporal interactions between excitation and inhibition on microsecond timescales are critical for binaural processing.

(COI: No)

MS03-4

Interplay between inhibition and voltage-gated K channels during binaural computations

Golding, Nace[1,2] ([1]*Univ.Texas, Austin, Texas, USA;* [2]*University of Leuven, Leuven, Belgium*)

Neurons in the mammalian medial superior olive (MSO) detect interaural time differences (ITDs), cues that reflect the location of sounds along the horizontal plane. Feedforward glycinergic inhibition has been proposed to control ITD detection but its role and mechanism of action are controversial. To understand how inhibition shapes binaural processing we made intracellular whole-cell recordings from gerbils in vivo and recorded responses to binaural stimuli. Application of the glycine receptor blocker strychnine through a second pipette increased spike rates and usually widened ITD functions or enhanced side lobes, but did not alter the best ITD. Using dynamic clamp and dual somatic recordings from MSO neurons in brain slices, we found that the interplay between fast glycinergic IPSPs and low voltage-activated K channels reduces distortions in the shape and timing of EPSPs. During inhibition, the sharpening of EPSPs due to inhibitory shunting was offset by the deactivation of low voltage-activated K channels that are expressed at high density in MSO neurons. The primary effect of inhibition was to reduce spike probability and sharpen ITD curves through an iceberg-type mechanism, consistent with in vivo results. We conclude that the interplay between inhibition and K channels provides a mechanism that enables inhibition to modify the resolution but not location of spatial receptive fields

(COI: No)

Meeting Symposium 4

NO, the subsequent evolution

(March 21, 17:00～18:30, Room F)

MS04-1

NO, the subsequent evolution

Maeda, Masanobu (*Dept Physiol, Wakayama Med Univ Sch Med, Wakayama, Japan*)

In the 1990s, the number of the papers related with NO (nitric oxide) had become very huge. In 1993, NO became Moleculara of the year. In 1998, Dr. F. Murad, Dr. L. J. Ignarro and Dr. R. F. Furchgott got Nobel prizes. The NO research had reached peak in 1990s, and almost twenty years have passed. We would like to discuss NO research at the present time in this symposium.

(COI: No)

MS04-2

Expression of nitric oxide synthase (NOS) in bone

Ambe, Kimiharu; Watanabe, Hiroki (*Div. Oral Histol., Dept. Morphol. Biol., Ohu Univ. Sch. Dent., Koriyama, Japan*)

Nitric oxide (NO) is a free radical which is produced from a wide variety of cells. NO is involved in the regulation of many physiological processes, such as vascular relaxation, neurotransmission, and immune regulation. NO is generated by nitric oxide synthase (NOS), which has three identified isoforms: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). Different isoforms are expressed depending on the organs, tissues, and cells, and investigation of the types and functions of enzymes expressed in various tissues is underway. We investigated the expression and cellular localization of NOS isoforms in mice calvaria and femur using immunohistochemistry and *in situ* hybridization to clarify their possible roles in bone. The immunoreactivity of eNOS was weakly positive in the osteoblasts of calvaria at 3 weeks of age, but immunoreactivity of nNOS or iNOS was not observed in the osteoblasts. In contrast, the immunoreactivity of nNOS, iNOS, and eNOS was positive at 3 weeks of age in femur in which bone matrix was formed. At 18 weeks of age, weakly positive reaction of nNOS and eNOS was also noted in osteoblasts, similarly to that at 3 weeks of age, but reaction of iNOS was negative. Expression of NOS mRNA for *in situ* hybridization showed results approximately similar to those of immunohistochemistry. On the other hand, in each NOS knockout mice, the expression levels of the remaining NOSs were increased. These results suggest that each isoform of NOS may be related to bone growth and remodeling in mice calvaria and femur.

(COI: No)

MS04-3

NO functions in plant defense responses against pathogen attack

Kawakita, Kazuhito (*Plant Pathol Lab, Grad Sch Bioagr, Nagoya Univ, Nagoya, Japan*)

In plants, nitric oxide (NO) and reactive oxygen species (ROS) play crucial roles in the regulation of various physiological processes. NO and ROS have been also shown to be an important messenger in plant defense signaling against microbial pathogens. They participate in the induction of resistance reactions such as the expression of defense-associated genes, accumulation of antimicrobial compounds (phytoalexins) and induction of hypersensitive cell death.

In most living organisms, NO is supposed to be mainly produced by nitric oxide synthase (NOS), which catalyzes NO and L-citrulline formation from O_2 and L-arginine. NOS-like activity in plants has been reported widely. However, it is speculated that there is no plant protein corresponding to mammalian NOS. Nitrate reductase, a key enzyme of nitrogen assimilation, is considered to be another enzyme that is capable of producing NO in plants.

We searched for compounds that elicit NO production in plants and found *bis*-arylmethanone compound. The compound elicited hypersensitive cell death but no phytoalexin in potato. Resistance against *Phytophthora infestans*, the potato late blight pathogen increased in potato leaves treated with the compound. In *Nicotiana benthamiana*, a model tobacco plant treated with the compound, NO production was induced without the induction of ROS or hypersensitive cell death. The compound also induced resistance in *N. benthamiana* against *P. infestans*. These results suggested that both NO production and ROS production are required for the induction of hypersensitive cell death.

(COI: No)

MS04-4

Redox regulation of soluble guanylate cyclase -from the point of vascular function study-

Tawa, Masashi; Okamura, Tomio (*Dept. Pharmacol., Shiga Univ. Med. Sci., Otsu, Japan*)

Nitric oxide (NO) plays an essential role in regulating vascular tone. This molecule activates soluble guanylate cyclase (sGC) by binding to its reduced (Fe^{2+}) heme moiety, leading to elevation of intracellular cGMP levels, but once the prosthetic heme moiety is oxidized (Fe^{3+}) or lost, NO loses its ability to stimulate sGC. As valuable tools for elucidating the redox state of sGC, two different types of compounds that act directly on sGC (sGC stimulators and sGC activators) have been recently utilized. sGC stimulators can activate the reduced form of sGC in the absence of NO, whereas sGC activators preferentially and effectively stimulate this enzyme when it is in the NO-unresponsible, heme-oxidized or heme-free state [Follmann et al., doi: 10.1002/anie.201302588]. In organ chamber experiments, exposure to the intracellular superoxide generator menadione impaired the relaxant response of endothelium-denuded rat iliac arteries to the sGC stimulator BAY 41-2272, whereas it augmented that to the sGC activator BAY 60-2770. Similar results were obtained in the arteries exposed to peroxynitrite. These influences of intracellular superoxide, but not of peroxynitrite, on the BAY compound-induced vasorelaxations were eliminated in the presence of tempol. On the other hand, hydrogen peroxide exposure did not affect the relaxation induced by either BAY 41-2272 or BAY 60-2770. These findings suggest that sGC redox equilibrium is shifted towards the NO-insensitive oxidized/heme-free state in the diseased blood vessel associated with the increase in certain reactive oxygen and nitrogen species level.

(COI: No)

MS04-5

Crucial Role of Endogenous and Exogenous NO Production Systems in the Pathogenesis of Cardiovascular and Metabolic Diseases

Tsutsui, Masato (*Department of Pharmacology, Graduate School of Medicine, University of the Ryukyus*)

Nitric oxide (NO) is endogenously synthesized by three distinct NO synthases (NOSs), all of which are expressed in the human body The roles of NO derived from all NOSs have been examined in pharmacological studies with non-selective NOS inhibitors. However, due to non-specificity of the NOS inhibitors, the authentic roles of NOSs-derived NO are still poorly understood. To address this issue, we developed mice in which all three NOS genes are completely disrupted. The triple NOSs null mice were not embryo-lethal; however, their fertility and survival were markedly reduced as compared with wild-type mice. The triple NOSs null mice exhibited a variety of phenotypes, including acute myocardial infarction, diastolic heart failure, and metabolic syndrome. These results suggest that endogenous NOSs deficiency leads to cardiovascular and metabolic diseases in mice. On the other hand, it has recently been discovered that NO is produced from NO metabolites, nitrite (NO_2^-) and nitrate (NO_3^-), the latter of which is rich in green leaf vegetables. Based on the background, we have recently elucidated that long-term exogenous NO_2^-/NO_3^- deficiency in a diet resulted in metabolic syndrome in mice, identifying the specific dietary ingredient that causes metabolic syndrome even in the absence of calorie excess. These findings provide the first evidence for the crucial role of endogenous and exogenous NO production systems in the pathogenesis of cardiovascular and metabolic disorders.

(COI: No)

Meeting Symposium 5

"La raison d'être" of the Associations, Councils, Committees and Unions of the Academic Societies

(March 22, 9:00〜10:30, Room A)

1. 日本学術会議
本間 さと　北海道大・医〈生理〉
岡部 繁男　東京大・医〈解剖〉

2. 日本医学会連合
加藤 総夫　慈恵医大〈生理〉
河田 光博　京都府医大〈解剖〉

3. 日本医学会連合用語管理委員会
松村 譲児　杏林大・医〈解剖〉
坂井 建雄　順天堂大・医〈解剖〉

4. 日本医学雑誌編集者会議
石川 義弘　横浜市大・医〈生理〉

5. 日本脳科学関連学会連合
伊佐 正　生理研〈生理〉
岡部 繁男　東京大・医〈解剖〉

6. 生物科学学会連合
仲嶋 一範　慶應・医〈解剖〉
小西 真人　東京医大〈生理〉

7. 総合討論

Meeting Symposium 6

Molecular mechanism and physiological function of cell polarity: through the function of transporters

(March 22, 17:30〜19:00, Room C)

MS06-1

The role of polarized transport in epithelial cells

Yoshimura, Shinichiro; Nakajo, Atsuhiro; Iwano, Tomohiko; Kunii, Masataka; Harada, Akihiro (*Grad Sch Med Osaka Univ., Osaka, Japan*)

The transport pathway in epithelial polarized cells is directed to apical or basolateral plasma membrane, which are distinct in protein and lipid composition. Several findings suggest that newly synthesized protein exported from the trans-Golgi network (TGN) is delivered to the endocytic recycling compartment (ERC), which is also regarded as recycling endosome, and sorted to apical or basolateral plasma membrane. Rab8 is thought to be a key molecule to regulate apical transport from ERC. In Rab8-knockout small intestinal cells, apical cargo proteins are misorted to lysosome. Here we identify a novel Rab8 interacting protein complex, which provides a mechanical insight into Rab8-regulated apical transport.

(COI: No)

MS06-2

Tight Junction-based Building of apical microtubule network of epithelial cells

Yano, Tomoki[1]; Matsui, Takeshi[2]; Tamura, Atsushi[1]; Uji, Masami[1]; Kanoh, Hatsuho[1]; Tsukita, Sachiko[1] ([1]*Lab Biol Sci, Grad Sch Med, Osaka Univ, Osaka, Japan;* [2]*Lab. Skin Homeostasis, RCAI, RIKEN Center for Integrative Med. Sci. Yokohama, Japan*)

Our body is wrapped by epithelial cell sheets. The structure of epithelial cell sheets, in which cell-cell adhesion is highly organized, is critically dependent on the association of actin filaments with apical cell-cell adhering junctions. However, relatively little is known of the roles of microtubules (MTs) during epithelial morphogenesis and cell-cell contact. Here, we found novel non-centrosomal MT networks, in which the sides of the MTs bundles were associated with TJs. Then, we identified that cingulin is a MTs-binding protein which were integrated in TJ. And we found that head-domain of cingulin bind to α-tubulin. The binding of head domain of cingulin to MTs was depends on phosphorylation of serine residues by AMPK which is sensitive to metabolic homeostasis-relate kinase. The dephosphomimetic mutant of head domain of cingulin bind to C-terminus of cingulin. Using the low angle shadowing technique to visualize cingulin molecule structures, it was revealed that the molecules of phosphomimetic and dephosphomimetic mutants formed the thread form and pills form, respectively. These data showed that the conformational regulation was related to the phosphorylation of head domain of cingulin. In addition, although wild-type colonies formed spheres in 3-D culture, the cingulin knocked-down cells had anisotropic shapes. These findings collectively suggest that the cingulin-regulated MTs association has a specific role in TJ-related epithelial morphogenesis.

(COI: No)

MS06-3

Identification of a new heterodimeric amino acid transporter in the apical membrane of renal proximal tubule

Nagamori, Shushi; Kanai, Yoshikatsu (*Dept Pharmacol, Grad Sch Med, Osaka Univ, Osaka, Japan*)

The heterodimeric amino acids transporter (HAT) family, which consists of heavy chains (SLC3) and light chains (SLC7) linked with a disulfide bond, has important physiological roles in many types of organs. The light chains have 12 transmembrane domains and transport amino acids. The heavy chains, which have one transmembrane domain and a large extra-cellular domain, are considered as accessory chains. While the light chains are nine molecules, only two molecules are known as heavy chains and distinguished by the localization in polarized cells. rBAT (SLC3A1) localizes at the apical side and 4F2hc/CD98hc (SLC3A2) expresses at the basolateral side. The heavy chains define where the heterodimers should work in polarized cells.
One of heavy chains, rBAT has been known to form a heterodimeric complex only with $b^{0,+}$AT (SLC7A9). Mutations in the two genes cause cystinuria, an autosomal recessive disorder of renal reabsorption of cystine at the apical membranes of proximal tubules. However, the expression paradox of these proteins in the kidney has been remaining. While rBAT highly express in the S3 segment of the proximal tubules, the expression of $b^{0,+}$AT decrease from the S1 to the S3 segment. Thus, an unknown partner of rBAT in the S3 segment has been suggested. By proteomics combined with biochemical analysis, we have identified SLC7A13 as a new rBAT partner. Furthermore, our findings strongly indicate that SLC7A13 is the secondary cystine transporter at the apical membrane of the late proximal tubules in the kidney, which was postulated the presence in 1970's.

(COI: No)

MS06-4

The unidirectional K^+-transport across the epithelial tissue in the inner ear establishes the endocochlear potential essential for hearing

Hibino, Hiroshi[1]; Nin, Fumiaki[1]; Murakami, Shingo[2]; Kurachi, Yoshihisa[2] (*[1]Dept. Mol Physiol, Niigata Univ Sch Med, Niigata, Japan; [2]Div Mol Cel Pharm, Dept Pharm, Grad Sch Med, Osaka University*)

Cochlear endolymph, an extracellular fluid containing 150 mM K^+, exhibits a positive potential of +80 mV. This called endocochlear potential (EP), which is essential for hearing, has been thought to be achieved by unidirectional K^+-transport through the lateral cochlear wall. However, the mechanism has been uncertain. The lateral wall comprises inner epithelial layer whose apical surface faces the endolymph, and outer layer, of which basolateral membranes are exposed to an ordinary extracellular fluid, perilymph. Each layer expresses K^+ channels apically and K^+-uptake transporters basolaterally. Intrastrial space (IS), an extracellular compartment between the two layers, exhibits low K^+ and a potential similar to the EP. By using electrodes sensitive to potential and K^+, we found that the positive IS potential (ISP) dominates the EP and represents a K^+-diffusion potential elicited by a large K^+-gradient across the apical surface of the outer layer. Mathematical approaches revealed that the unidirectional K^+-transport underlies the K^+-gradient and depends on the function and polarized localization of the channels and transporters. Finally, ischemia and an ototoxic drug, which block the basolateral K^+-transporters in the inner layer, disrupt the unidirectional K^+-transport and diminish the K^+-gradient, leading to loss of the ISP and EP.

(COI: No)

Meeting Symposium 7

Neural development and neuropsychiatric disorder models

(March 23, 9:00～10:30, Room C)

MS07-1

A mouse model for 15q duplication towards understanding the pathophysiology of autism

Takumi, Toru (*RIKEN BSI, Wako, Japan*)

Autism is a complex psychiatric illness that has received considerable attention as a developmental brain disorder. Substantial evidence suggests that chromosomal abnormalities including copy number variations contribute to autism risk. The duplication of human chromosome 15q11-13 is known to be the most frequent cytogenetic abnormality in autism. We have modeled this genetic change in mice using chromosome engineering to generate a 6.3-Mb duplication of the conserved linkage group on mouse chromosome 7. Mice with a paternal duplication display autistic-like behavioral features such as poor social interaction and stereotypical behavior, and exhibit abnormal ultrasonic vocalizations. This chromosome-engineered mouse model for autism seems to replicate various aspects of human autistic phenotypes and validates the relevance of the human chromosome abnormality. This model is a founder mouse for forward genetics of a developmental brain disorder and an invaluable tool for its therapeutic development. I will present our analyses on these mice towards understanding the molecular pathophysiology of autism spectrum disorders.

(COI: No)

MS07-2

Dysregulation of RNA metabolism potentially causes neurodegenerative and developmental neuropsychiatric disorders

Takeuchi, Akihide[1]; Iida, Kei[1]; Ninomiya, Kensuke[1]; Tsubota, Tomoaki[1]; Denawa, Masatsugu[1]; Itou, Mikako[2]; Ohno, Kinji[2]; Hagiwara, Masatoshi[1] (*[1]Grad.Sch.Med.Kyoto Univ., Kyoto, Japan; [2]Grad.Sch.Med.Nagoya Univ., Nagoya, Japan*)

In mammals, several essential neuronal genes are expressed from extra-long pre-mRNAs, which are vulnerable to dysregulation during transcriptional elongation or processing. Several neurodegenerative and neuropsychiatric disorders are thought to be caused by the impairment of extra-long pre-mRNA expression. However, the identities and functions of essential molecules facilitating long neuronal gene expression are still largely unknown. Here, we discovered a novel function of the RNA-binding protein Sfpq in extra-long neuronal gene expression. Genome-wide Sfpq-binding mapping and transcriptome analyses showed that Sfpq binds co-transcriptionally across the entire span of pre-mRNAs of long genes and is required for the expression of pre-mRNAs > 100 Kbp. RNA polymerase II density analysis revealed that Sfpq is necessary for transcriptional elongation beyond 100 Kbp. Our findings using Sfpq-mutant mice indicated that Sfpq-dependent transcription of long genes is essential for neuronal development and that dysregulation of long genes potentially causes neurodegenerative and developmental neuropsychiatric disorders.

(COI: No)

MS07-3

The pathomechanism of Huntington disease: factors related to its pathological cascades

Nukina, Nobuyuki (*Juntendo University Graduate School of Medicine*)

Huntington Disease (HD) is a dominantly inherited neurodegenerative disorder caused by the accumulation of mutant huntingtin protein(HTT) containing an expanded polyglutamine (polyQ) tract. Most well established model mouse for HD is R6/2, transgenic mouse of HTT exon1 and we also established transgenic mouse of HTT exon1 with EGFP. Using those mice, we have been analyzing the pathomechanism of polyglutamine disease and searching the therapy for this disorder. We focused on nuclear inclusions, the main pathology of polyglutamine disease and polyglutamine aggregate interacting proteins(AIPs). AIPs include proteasome subunit, molecular chaperons, autophagy related molecules, RNA binding proteins and transcriptional factor. In this talk, I will introduce the pathological significance of those AIPs and recent discovery of new anatomy of basal ganglia based on the distribution of Scn 4b, which expresses in the striatum and decreased in HD.

(COI: No)

MS07-4

Synaptic defect in non syndromic autism: from the study in Neuroligin-3 mutant mice

Tabuchi, Katsuhiko[1,2] (*[1]Dept Mol Cell Physiol, Shinshu Univ Sch Med, Matsumoto, Japan; [2]PRESTO JST*)

Neuroligins and Neurexins are distinct families of cell adhesion molecules localized at post- and pre-synaptic terminals, respectively. They bind each other at synaptic cleft via their extra cellular domains and induce synapse maturation. R451C mutation in neuroligin-3 is the first identified neuroligin mutation that had been shown to affect the surface localization of Neurolign-3 protein by in vitro studies. We generated knock-in mice that recapitulate this mutation to examine its relevance to autism. These mice grew normally without exhibiting obvious physical phenotypes but showed behavioral abnormalities relevant to autism including impaired social interaction and enhancement of spatial learning and memory. We studied synaptic function of these mutant mice and found inhibitory synaptic transmission was selectively enhanced in the cerebral cortex. Administration of GABA receptor blocker ameliorated the impaired social interaction suggesting this mutation could be the cause of autistic behavior in these mice. We further found ratios of NMDA/AMPA and NR2B/NR2A, and synaptic plasticity were increased in hippocampus indicating synaptic maturation was impaired in these mice. We hypothesized that disturbance of synaptic maturation causes impairment in social behavior and extraordinary memory ability in certain type of autism patients.

(COI: No)

Meeting Symposium 8

Frontiers in biological application of microscopic measurements

(March 23, 9:00～10:30, Room G)

MS08-1

Dynamic property of transcription factors analyzed by fluorescence cross-correlation spectroscopy in living cells

Kinjo, Masataka (*Grad.Sch.Adv. Life Sci. Hokkaido Univ. Sapporo, Japan*)

Two-laser-beam fluorescence cross-correlation spectroscopy (FCCS) is promising technique that provides dynamic and quantitative information about the interactions of biomolecules. By using FCCS, we determined the dissociation constant (Kd) of the p50/p65 heterodimer, homodimer of that, and also homodimer of glucocorticoid receptor (GR) in living cells. GR is a well-known steroid-dependent nuclear receptor protein. The classical view is as follows; unliganding GR resides in the cytoplasm, translocates to the nucleus upon ligand binding, and then associates with a specific position, namely glucocorticoid response elements (GRE), where GR act as a transcription factor. On the other hand, it is still a puzzle whether GR forms a dimer in the cytoplasm or in the nucleus before DNA binding or after that. Our result using FCCS suggested that high values of Kd in nucleus and so the dimer-monomer equivalent state in the nucleus and also cytoplasm. These findings support the existence of a "dynamic monomer pathway"and regulation of GR function can be controlled by concentration and balance of monomer and dimer ratio.

(COI: No)

MS08-2

Measuring the distribution of small molecule compounds inside biological tissue via intrinsic molecular vibrations using nonlinear Raman spectroscopy

Kawagishi, Masahiko[1]; Obara, Yuki[2]; Suzuki, Takayuki[2]; Hayashi, Masumi[3]; Misawa, Kazuhiko[2]; Terada, Sumio[1] ([1]*Dept.Neuroanat.Cell.Neurobiol., Grad.Sch. Med.Dent.Sci., Tokyo Med.Dent.Univ., Tokyo, Japan;* [2]*Dept.Appl.Phys., Tokyo Univ. Agricul.Technol., Koganei, Japan;* [3]*Wired Co., Ltd., Komae, Japan*)

Distributions of small molecular weight (less than 300 Da) organic compounds inside biological tissue have been obscure because of the lack of appropriate methods to measure them. Raman spectroscopy is a technique to study molecular vibrational signature, specific to the chemical bonds and symmetry of the molecule. It can acquire information about chemical compounds without any labeling. Coherent anti-Stokes Raman scattering (CARS) is a third-order nonlinear optical process to produce a coherent and stronger Raman signal. We have been using CARS spectroscopy to detect and identify small molecule compounds. We have developed a time-resolved and phase-sensitive technique to remove non-resonant noise signals from water and to acquire resonant vibrational CARS signals from a target compound in aqueous environment. We applied this technique to detect small molecular weight drugs inside biological tissue. As an initial model experiment, we measured sevoflurane in squid giant axon. We also measured taurine inside mouse cornea and successfully characterized its depth profile. Our CARS spectra measurement can be a promising method to measure and visualize the distribution of small bio-related compounds in biological background without using any labeling, paving the way for new cell biological analysis in various disciplines.

(COI: No)

MS08-3

Dynamics of protein assemblies in live cells revealed by monitoring orientation of individual molecules

Tani, Tomomi[1,2] ([1]*Marine Biological Laboratory, Woods Hole, Massachusetts, USA;* [2]*Dartmouth College*)

Monitoring orientation of individual molecules has yielded new insights in the structure and function of proteins and the assemblies. Current fluorescent single molecule methods are limited to measurement of orientation of single fluorophores and little has permitted robust imaging of orientation of multiple molecules simultaneously in living cells. We have developed a new fluorescence polarization microscope that instantaneously and efficiently sorts the emitted fluorescence along four polarization orientations to provide instantaneous imaging of position and orientation of single fluorescent molecules and their assemblies. The instantaneous imaging of fluorescence polarization has enabled analysis of molecular position and orientation in vitro and in living cells. Taking advantage of Alexa Fluor 488 phalloidin that reports local orientation of actin filaments, we have measured changes in the orientation of local actin filaments as they undergo retrograde flow at the leading edge of migrating human keratinocytes. We also used our system to study organization of septins, a highly conserved cytoskeleton critical for cytokinesis and intracellular compartmentalization. We found that individual septin molecules with constrained GFP in filamentous fungus bind to the cell cortex with consistent orientation with respect to the cell axis. Our single molecule fluorescence orientation imaging technique in living cells is also promising to explore conformational changes in single molecules or mechanisms of protein assembly.

(COI: No)

MS08-4

Subnanometer-scale measurements at solid/liquid interfaces by frequency modulation atomic force microscopy

Fukuma, Takeshi[1,2]; Asakawa, Hitoshi[2]; Inada, Natsumi[1]; Takao, Kazufumi[1]; Miyata, Kazuki[1]; Miyazawa, Keisuke[1] ([1]*Kanazawa Univ., Kanazawa, Japan;* [2]*Bio-AFMCenter, Kanazawa, Japan*)

Frequency modulation atomic force microscopy (FM-AFM) has been traditionally used for atomic-scale imaging of various materials in vacuum. Recently, we have developed liquid-environment FM-AFM that is capable of imaging atomic-scale surface structures at solid-liquid interfaces. With the developed technique, we have demonstrated subnanometer-scale imaging of biological systems in physiologically relevant solution. Furthermore, we recently developed a three-dimensional (3D) force measurement technique referred to as 3D scanning force microscopy (3D-SFM). We applied this technique to visualize 3D force distribution at a mica-water and lipid-water interface. The obtained force image shows atomic-scale contrasts that represent a 3D water density distribution (i.e. hydration structure) as well as spatial distribution of flexible surface structures such as lipid headgroups. The results demonstrate the possibility of 3D imaging of 3D hydration and flexible surface structures at a solid-liquid interface.In this talk, I will present a short summary of liquid-environment FM-AFM instrumentation and its applications to the investigations on soft interfacial structures.

(COI: No)

Meeting Symposium 9

Leading-edge of science advanced by new electron microscopic technology for 3D reconstruction

(March 23, 15:00〜16:30, Room G)

MS09-1

The principle and applications of 3-D subcellular morphological analyses by Serial block-face scanning electron microscopy (SBF-SEM)

Miyazaki, Naoyuki; Murata, Kazuyoshi (*NIPS, Japan*)

Elucidating the spatial distribution of organelles and molecules within a whole cell is critical for understanding the nature of cellular processes, and whole-cell structural analysis at a high resolution is necessary for precise structural interpretation. SBF-SEM is an advanced 3-D electron microscopy technique for investigating such large volumes at a resolution of a few tens of nanometeres. In this method, thin surface of a resin embedded specimen is cut off by a diamond knife attached to an in-chamber ultramicrotome, and then the newly exposed surface is imaged by SEM. The sectioning and imageing are automatically repeated to get a serial block-face images of the specimen. 3-D structure is reconstructed from the serial block-face images after image alignment. In this presentation, we will show the principle and the performance of SBF-SEM, and introduce some recent applications. For example, morphological changes in mitochondria regulated by Cdc48p/p97 ATPase was examined by SBF-SEM (Miyazaki et al., 2014). Cdc48p is a highly conserved cytosolic AAA chaperone that is involved in a wide range of cellular processes and regulates mitochndrial morphology. The 3-D morphological analyses of SBF-SEM showed that loss of the ATPase activity of Cdc48p leads mitochondrial fragmentations and aggregations without fusion of the mitochondrial outer membrane, which suggested that Cdc48p is involved in the mitochondrial fusion process. Our results demonstrate that SBF-SEM has considerable advantages in morphological and quantitative studies on organelles and intracellular structues in entire cells.

(COI: No)

MS09-2

Serial section scanning electron microscopy and its application for the morphological analysis of the Golgi apparatus

Koga, Daisuke; Kusumi, Satoshi; Ushiki, Tatsuo (*Niigata Uni. Grad. Sch. Med. Dent. Sci., Niigata, Japan*)

Serial section scanning electron microscopy (SEM) is based on the collection of backscattered electron images of serial ultrathin sections on solid substrates such as glass slides. This method is simpler than serial section transmission electron microscopy, and is useful for 3D reconstruction of cellular structures without using special instruments such as focused ion beam/SEM (FIB/SEM) or serial block face/SEM (SBF/SEM). Serial section SEM has a number of advantages in the following points. (1) it is free from handling of the grid. (2) Sections can be imaged repeatedly. (3) An extensive area of a specimen can be observed. Here, we will explain details of serial section SEM, and show its application for the 3D morphological analysis of the Golgi apparatus. This technique is suitable for 3D reconstruction of the Golgi apparatus which is known as a cell organelle with a complicated structure. In this study, we will introduce the entire shape of the Golgi apparatus in different cell types. The shape of the Golgi apparatus was various depending on cell types, and appeared as a single mass of Golgi cistern networks. The combination of serial section SEM and immunohistochemistry is expected to be used for clarifying the 3D structure of the Golgi apparatus in relation to its function.

(COI: No)

MS09-3

Analysis of synaptic connectivity with FIB/SEM: antidepressant-induced morphological changes in perforant path synapse in the dentate gyrus

Kitahara, Yosuke[1]; Ohta, Keisuke[2]; Sotogaku, Naoki[1]; Nakamura, Keiichiro[2]; Nishi, Akinori[1] (*[1]Dept of Pharmacol, Kurume Univ Sch of Med, Kurume, Japan; [2]Dept of Anatomy, Kurume Univ Sch of Med, Kurume, Japan*)

Neural circuit is composed of many neurons, which communicate each other via synaptic connections. When profiling neural circuits, it is important to visualize the details of synaptic connection (e.g., dendritic spine and presynaptic bouton), which are related to neural activity. For analysis of synaptic connection, high resolution imaging at the electron microscopy (EM) level is required. Focused ion beam (FIB)/scanning electron microscopy (SEM) can automatically obtain serial EM images, and 3D reconstruction of synaptic morphology in multiple samples becomes possible. By utilizing advanced technology of FIB/SEM, we investigated the fluoxetine-induced morphological changes in perforant path synapse in the middle molecular layer of DG, in which synaptic transmission is enhanced after chronic fluoxetine treatment. 3D analyses of dendritic spines revealed the appearance of the extremely large-sized spine after chronic fluoxetine treatment. The presynaptic bouton connected to the large-sized spine was large in volume, and the volumes of mitochondria and synaptic vesicle in the bouton were correlated with sizes of bouton. The fluoxetine-induced increases in sizes of pre- and post-synaptic structures of perforant path synapse may be involved in the enhanced glutamatergic neurotransmission. Thus, 3D image analysis with FIB/SEM is useful to visualize morphological features of synaptic connection under pathophysiological conditions.

(COI: No)

MS09-4

Space–time wiring specificity supports direction selectivity in the retina

Kim, Jinseop S.[1†]; Greene, Matthew J.[1]; Zlateski, Aleksandar[2]; Lee, Kisuk[1]; Richardson, Mark[1]; Turaga, Srinivas C.[1]; Purcaro, Michael[1]; Balkam, Matthew[1]; Robinson, Amy[1]; Behabadi, Bardia F.[3]; Campos, Michael[3]; Denk, Winfried[4]; Sebastian, Seung H.[1†]; the EyeWirers[5] (*[1]Departments of Brain and Cognitive Sciences; [2]Electrical Engineering and Computer Science, MIT, USA.; [3]Qualcomm Research, USA.; [4]MPI at Heidelberg, Germany.; [5]http://eyewire.org. [†]Present address: Princeton Neuroscience Institute, Princeton University, USA*)

What is the origin of neural computation? There are two possible theories. In biophysics, a neuron is considered to have internal mechanism that makes itself an already capable computer. The connectionism, on the other hand, claims that it is rooted in the connections between neurons. I will present a recent finding that supports the connectionism from a study about motion detection in the mammalian retina. The starburst amacrine cells (SACs) are known to exhibit direction selectivity (DS) and play crucial roles for DS of other neurons as well, yet the origin of their DS remains elusive. In search of clues, we reconstructed Off-type SACs and bipolar cells (BCs) in serial electron microscopic images with help from EyeWire, an online community of 'citizen neuroscientists'. We found quantitative evidence that one BC type prefers to wire with a SAC dendrite near the SAC soma, whereas another BC type prefers to wire far from the soma. The near type is known to lag the far type in time of visual response. A mathematical model shows how such 'space–time wiring specificity' could make SAC dendrites respond selectively to stimuli that move in the outward direction from the soma.

(COI: No)

Committee Symposium

Committee Symposium 1

Current Status and Issue of Research Ethics

(March 21, 8:30～10:00, Room B)

CS01-1 Roles of Academia and Research Society in Ethical Education for Young Scientists

Kurata, Kiyoshi Dept Physiol, Hirosaki Univ Grad Sch Med, Hirosaki Japan

CS01-2 Pitfalls in modern scientific research: neglectfulness and ethics

Takeda, Sen Univ.Yamanashi. Fac.Med.

CS01-3 What we can do not to repeat fraudulence in science

Honma, Sato Dept. Chronomedicine, Grad.Sch Med, Hokkaido Univ. Sapporo, Japan

CS01-4 Misconduct and university management

Takata, Kuniaki Gunma Univ, Maebashi, Japan

Committee Symposium 2

Brain structures from physiological viewpoints; brain functions from anatomical viewpoints

(March 21, 8:30～10:00, Room F)

CS02-1

Regulation of telencephalic development through glycogen

Gotoh, Hitoshi (*Kyoto Pref. Univ. Med., Kyoto, Japan*)

Cellular metabolism has reported to be involved in cell proliferation and differentiation. Mammalian embryos faces 'nutritional shift' on their birth because of the separation from placental energy supply. However, it remains unclear how the cellular metabolism is changed and maintained in the brain. Glycogen is a branched polysaccharide and act as energy stores that rapidly supply energy depending on the demand. Glycogen is reported to be present in developing brain, however, the function of glycogen in the developing brain remains unclear. In order to analyze the function of glycogen in the central nervous system, we first analyzed localization of glycogen in the developing telencephalon. We found that glycogen particles are abundantly present in the border of ventral/dorsal telecephalon. GLAST-positive glial lineage cells but not neuronal cells have glycogen inside the cell. Further, we found that quantity of glycogen is dramatically decreased after birth. In addition, glycogen phosphorylase, a glycogen degrading enzyme, is activated as compared with that of embryonic stage. To analyze its functions, we injected inhibitors of glycogen phosphorylase, and found that inhibition of glycogen phosphorylase lead to decreased cell proliferation. In primary cultured astrocytes, p21cip, a cell cycle inhibitor, was upregulated and cells were arrested at G1-S phase upon glycogen phosphorylase inhibition. These results suggest that glycogen is a energy source that is required for maintaining cell proliferation upon transition of embryonic to postnatal stage.

(COI: No)

CS02-2

Molecular mechanism underlying development of the cerebral cortex -Roles of Dpy19 family-

Watanabe, Keisuke (*Div Neurobiol Anat, Niigata Univ*)

The mammalian cerebral cortex has been evolutionarily expanded and acquired a distinct six-layer structure. However, developmental mechanisms specific to this area are poorly understood. Recently, we have revealed that a multi-transmembrane protein Dpy19L1 is required for proper migration of glutamatergic neurons in the developing cortex. In mammals, Dpy19 family consists of four members (Dpy19L1-L4), which have been revealed to be associated with human diseases. However their functions during development have been unclear. In this study, we examined roles of Dpy19 family members in the developing cerebral cortex. Dpy19L1, Dpy19L3 and Dpy19L4 showed distinct expression patterns in the developing cortex. Furthermore, we have generated Dpy19L1, Dpy19L3 and Dpy19L4 mutant mice. A large number of Dpy19L1 homozygotes displayed postnatal lethality. Some Dpy19L1 knockout mice are viable, but smaller in size. Cortical layer formation was apparently normal in Dpy19 knockout brains. Interestingly, Dpy19 homozygotes showed weaker fear responses to predator odours, compared with that of wild-type and the heterozygous mice. Furthermore, we examined molecular functions of Dpy19 protein. Dpy19 is predicted to have multi-transmembrane domains, but lacks any predicted functinal domains. Our in vitro studies suggest a possible association of Dpy19L1 with the endoplasmic reticulum and microtubules. These results suggest important roles of Dpy19L1 in cortical development and innate fear responses.

(COI: No)

CS02-3

Changes of brain function and structure involved in functional recovery after brain damage

Higo, Noriyuki (*Systems Neurosci Sect, Human Tech Res Inst, AIST, Tsukuba, Japan*)

The central nervous system has the capacity for functional recovery following the damage. We previously reported that motor training after lesioning of the primary motor cortex (M1) induces recovery of dexterous hand movements including precision grasp in macaque monkeys (Murata et al., 2008). To clarify the neuronal bases underlying the functional recovery of dexterous movements after M1 lesion, we measured brain activity during performing a precision grasp task using positron emission tomography (PET). The PET imaging analysis revealed overactivity of the ventral premotor cortex (PMv) during the early post-recovery period (the period just after recovery) and increased functional connectivity within M1 during the late post-recovery period (the period at several months after recovery). The causal role of these areas in motor recovery was confirmed by means of pharmacological inactivation by muscimol during each recovery period. We also investigated the structural changes of neurons in the corresponding areas during the functional recovery using histochemical analysis; gene expression of growth-associated protein-43 (GAP-43) was increased in both the PMv during the early-post recovery period and the perilesional M1 during the late-post recovery period, suggesting that GAP-43-mediated structural changes of presynaptic axon terminals occurred in these areas. These findings indicate that in both the remaining primary motor and premotor cortical areas, time-dependent plastic changes, both functional and structural, are involved in functional recovery from the motor deficit caused by the M1 lesion.

(COI: No)

CS02-4

Type 1 metabotropic glutamate receptor regulates experience-dependent maintenance of mature synaptic connectivity in the visual thalamus

Narushima, Madoka[1]; Uchigashima, Motokazu[2]; Harada, Takeshi[3]; Hashimoto, Kouichi[4]; Aiba, Atsu[3]; Watanabe, Masahiko[2]; Miyata, Mariko[1,5]; Kano, Masanobu[6] (*[1]Dept Physiol, Sch Med, Tokyo Women's Medical Univ, Tokyo, Japan; [2]Dept Anatomy, Grad Sch Med, Hokkaido Univ, Sapporo, Japan; [3]Lab Animal Resources, CDBIM, Fac. Med, Univ. Tokyo, Tokyo, Japan; [4]Dept Neurophysio, Grad Sch Biomed & Health Sci, Hiroshima Univ, Hiroshima, Japan; [5]PRESTO, JST, Kawaguchi, Japan; [6]Dept Neurophysiol., Grad Sch Med, Univ Tokyo, Tokyo, Japan*)

In the dorsal lateral geniculate nucleus (dLGN), retinogeniculate (RG) synapses undergo formation and elimination during development and mature by postnatal day 20 (P20). Afterwards, visual experience is critical to maintenance of established RG synapses since one week of visual deprivation from P20 triggered remodeling of RG synaptic connectivity. In contrast to synapse formation and elimination phases, molecular basis of experience-dependent maintenance remained poorly understood. We found that expression of type 1 metabotropic glutamate receptor (mGluR1) increased in the dLGN after eye opening. In mGluR1 knock-out (KO) mice, formation and elimination of RG synapses was normal by P20 but the synapses were remodeled in the mice older than P28. Visual deprivation (p20-28) could not induce the remodeling in the KO mice. Importantly, in wild-type mice, pharmacological blockade of mGluR1 in the dLGN after P21 triggered remodeling of RG synapses and activation of mGluR1 in the dLGN rescued visual deprivation-induced remodeling. These results suggest that mGluR1 is required for the experience-dependent maintenance to conserve mature RG connectivity.

(COI: No)

Committee Symposium 3

Symposium by the Committee on the Promotion of Gender Equality

(March 21, 12:00～13:00, Room G)

CS03-1 Find the pride, joy and appreciation in life and work

Tokuda, Nobuko Grad. Sch. Med. Yamaguchi Univ., Yamaguchi, Japan

CS03-2 Changing times, Changing gender roles : Who do we want female researcher to be

Miyata, Mariko Tokyo womens medical univ.

Committee Symposium 4

Functional architecture of localization and integration of subcellular Ca^{2+} signaling

(March 21, 14:00～17:00, Room C)

CS04-1

Piezo1 integration of vascular architecture with physiological force

Beech, David J; Li, Jing; Hou, Bing (*Div Cardiovasc Res, Sch Med, Univ Leeds*)

Endothelial cells are strikingly sensitive to shear stress, a frictional force caused by blood flow. Vascular development depends on it, production of nitric oxide is strongly promoted by it, the location and size of atherosclerotic plaques depend on differences in shear stress imposed by vascular architecture, and shear stress-induced migration is pivotal in angiogenesis and wound healing. The nature of the sensor has nevertheless been elusive. We recently revealed a key player (Li et al 2014 Nature doi: 10.1038/nature13701). Piezo1 was important for normal shear stress-evoked calcium signalling and non-selective cationic current in endothelial cells. Piezo1 disruption in the mouse was embryonic lethal just at the time when vascular maturation was required from embryonic day 9.5. Lethality reflected specific requirement for endothelial Piezo1 because endothelial-specific disruption of Piezo1 led to endothelial cells which were present but which did not remodel to form mature vascular architecture. Adult haploinsufficient Piezo1+/- mice showed disturbed phosphorylation of endothelial nitric oxide synthase and reduced alignment of endothelial cells to the direction of blood flow. Downstream of Piezo1-dependent calcium signalling was protease (calpain) activation and spatial reorganization of endothelial cells to the polarity of the applied force. Piezo1 is suggested to have major significance in vascular biology and to be critical for the development of complex life.Supported by the Medical Research Council, Wellcome Trust, the British Heart Foundation, and Cancer Research UK. The author declares no conflict of interest.

(COI: No)

CS04-2

Genetically-encoded tools to optically control and image Ca^{2+} dynamics

Nagai, Takeharu (*ISIR, Osaka Univ, Japan*)

In living organism, Ca^{2+} is one of the most versatile second messenger to control biological processes such as muscle contraction, hormonal secretion and apoptosis induction. Its spatial and temporal dynamics has key roles to regulate these physiological phenomena. To reveal such dynamics, variety of Ca^{2+} indicators had been developed. They enabled noninvasive visualization of Ca^{2+} dynamics, provided meaningful information for research in wide range of biological field. However, for deeper understanding of relationship between the spatiotemporal Ca^{2+} dynamics and the following response, development of tools to manipulate intracellular Ca^{2+} level have been desired. For this, we developed a genetically-encoded photoactivatable Ca^{2+} releaser called PACR. That is composed of a Ca^{2+}+ binding protein and a light-sensitive protein LOV2 derived from phototoropin. Affinity of PACR for Ca^{2+} was decreased during irradiation of blue light but increased after the irradiation. Thus reversible and repeatable manipulation of Ca^{2+} concentration is possible without damages to living specimens. By using PACR, we succeeded nucleus specific temporal Ca^{2+} increase in HeLa cells and excitation of specific neuron in freely moving *C. elegans* by blue light irradiation. This useful tool is expected to contribute on researches to reveal the role of Ca^{2+} dynamics in complex biological phenomena. In addition to this manipulation tool, I would like to introduce cyan and orange color variants of bright luminescent Ca^{2+} indicators, which can be used compatibly with optogenetic actuators including PACR.

(COI: No)

CS04-3

Cellular processes mediated by a lipid-metabolizing enzyme diacylglycerol kinase (DGK) family

Goto, Kaoru (*Yamagata Univ. Sch. Med., Japan*)

Diacylglycerol kinase (DGK) phosphorylates a lipid second messenger diacylglycerol (DG) to phosphatidic acid and is involved in a variety of pathophysiological cellular responses through the metabolism of DG. DGK consists of a family of isozymes, each of which has a unique character in terms of regulatory mechanism, binding partner, and subcellular localization. Of DGKs, DGKzeta localizes primarily to the nucleus in various cell types. Under pathological conditions, DGKzeta translocates from the nucleus to the cytoplasm in hippocampal neurons in animal models of excitotoxicity. DGKzeta cytoplasmic translocation is shown to recapitulate in acute hippocampal slices exposed to oxygen-glucose deprivation (OGD), in which NMDA receptor-mediated Ca influx triggers this phenomenon. What is the functional implication of cytoplasmic translocation of DGKzeta? The transcription factor p53 plays a crucial role in coordinating the cellular responses to various stresses, such as apoptotic cell death. We found that DGKzeta physically interacts with p53. In addition, cytoplasmic DGKzeta is revealed to attenuate p53-mediated cytotoxicity against DNA damage by facilitating cytoplasmic anchoring and degradation of p53 through a ubiquitin-proteasome system. Concomitantly, decreased levels of nuclear DGKzeta engender down-regulation of p53 transcriptional activity. These findings suggest that DGKzeta cytoplasmic translocation is a protective stress response and attenuates p53-mediated cytotoxicity under stress conditions.

(COI: No)

CS04-4

Ca^{2+}- and Calmodulin-mediated regulation of receptor-operated cation currents of TRPC6 channels

Mori, Masayuki X[1]; Hirano, Mitsuru[1]; Hase, Hideharu[1]; Itsuki, Kyohei[2]; Inoue, Ryuji[3]; Mori, Yasuo[1] (*[1]Dept SynBiol, Grad Sch Engr, Kyoto Univ, Kyoto, Japan; [2]Sch Dent. Kyushu Univ, Fukuoka; [3]Sch Med, Dept Physiol, Fukuoka Univ. Fukuoka*)

Calmodulin (CaM) contributes a variety of ion channels gating regulation in response to cellular Ca2+ ([Ca2+]i) changes. However, the information is still missing about the molecular basis of CaM-mediated regulation of mammalian TRP channels which generate receptor-operated cation (Ca2+ and Na+) currents (ROC). To accumulate of Ca2+ and CaM roles in TRP channels, we first characterized the Ca2+ regulation in TRPC6 channels. The decay of the ROC of TRPC6 was delayed by chelation with EGTA or BAPTA, thus suggesting a global Ca2+ mechanism. We then examined CaM binding to the C-terminal region of TRPC6 by Ca2+-dependent FRET system. FRET due to CaM binding to the C-terminal region of TRPC6 demonstrated a bell-shape response curve with respect to [Ca2+]i. This Ca2+-dependence was a unique compared to those of IQ-domain of voltage-gated Ca or Na channels. The bell-shape response changed to a simple grow by a mutation in either N- or C-lobe domain of CaM. Intriguingly, the mutant in the N-lobe of CaM delayed the decay of receptor-operated currents of TRPC6, indicating the lobe-specific function. From these results, the Ca2+-dependent regulation of TRPC6 can be explain by the bell-shape response curve of CaM binding which is probably caused by a competitive binding between the both lobes of CaM. Our results provide a unique molecular basis for CaM to terminate ion channel activity, which may play critical roles at the down-stream of vasoconstrictors and growth factors.

(COI: No)

CS04-5

Imaging Intraorganellar Ca^{2+} at Subcellular Resolution Using CEPIA

Suzuki, Junji[1]; Kanemaru, Kazunori[1]; Ishii, Kuniaki[2]; Ohkura, Masamichi[3]; Okubo, Yohei[1]; Iino, Masamitsu[1] (*[1]Dept. Pharmacol., Grad. Sch. Med., Univ. Tokyo, Tokyo, Japan; [2]Dept. Pharmacol., Grad. Sch. Med., Yamagata Univ., Yamagata, Japan; [3]Brain Sci. Inst., Saitama Univ., Saitama, Japan*)

The endoplasmic reticulum (ER) and mitochondria accumulate Ca^{2+} within their lumens to regulate numerous cell functions. However, determining the dynamics of intraorganellar Ca^{2+} has proven to be difficult. We generated a family of genetically-encoded Ca^{2+} indicators, named calcium-measuring organelle-entrapped protein indicators (CEPIA), which can be utilized for intra-organellar Ca^{2+} imaging. CEPIA, which emit green, red or blue/green fluorescence, are engineered to bind Ca^{2+} at intra-organellar Ca^{2+} concentrations. They can be targeted to different organelles and may be used alongside other fluorescent molecular markers, expanding the range of cell functions that can be simultaneously analyzed. The spatiotemporal resolution of CEPIA makes it possible to resolve Ca^{2+} import into individual mitochondria while simultaneously measuring ER and cytosolic Ca^{2+}. We have used these imaging capabilities to reveal differential Ca^{2+} handling in individual mitochondria. Thus, CEPIA enable to study the physiological functions of intraorganellar Ca^{2+} dynamics.

(COI: No)

CS04-6

Local calcium signaling in neuronal development and remodeling

Emoto, Kazuo (*Dep Biol, Grad Sch Sci, Univ of Tokyo, Japan*)

Nervous system development relies on a balance between progressive and regressive events. After progressive events such as axon/dendrite outgrowth and synapse formation, neurons refine their connections through regressive events such as pruning of axons and dendrites. Thus, proper dendrite pruning critically depends on local activation of the elimination machinery in unwanted dendrites, but our understanding of locally acting mechanisms involved in this process remains incomplete. We have been working on how neurons can selectively eliminate unnecessary dendritic branches using Drosophila sensory neurons as a model system, and found that compartmentalized calcium transients in dendritic branches act as temporal and spatial cues to trigger pruning. By performing long-term in vivo imaging, we show that calcium transients occur in dendritic branches, but not in the soma or axon which exhibits no pruning, at ~3 hours prior to branch elimination. The compartmentalized calcium transients are induced in part by a local increase of dendritic excitability, which thereby activates calcium influx via voltage-gated calcium channels (VGCCs); blockade of VGCC activity impairs dendrite pruning. Further genetic analyses suggest that the calcium-activated protease calpain functions downstream of the calcium transients to promote dendrite pruning. Our findings reveal the importance of compartmentalized sub-dendritic calcium signaling in spatio-temporally selective elimination of dendritic branches.

(COI: No)

Committee Symposium 5

Japan-Korea Joint Symposium - Towards FAOPS2019

Morphological and Physiological Approaches to Synaptic Transmission

(March 22, 9:00～10:30, Room E)

CS05-1

Cellular mechanisms for mossy fiber input-induced heterosynaptic plasticity at direct cortical synapses in the hippocampal CA3 pyramidal cells

Lee, Suk-Ho; Hyun, Jung Ho; Eom, Kisang; Lee, Kyu-Hee; Ho, Won-Kyung (*Department of Physiology, Seoul National University College of Medicine, Seoul, Korea*)

A short high frequency stimulation of mossy fibers (MFs) induces long-term potentiation (LTP) of direct cortical or perforant path (PP) synaptic inputs in the hippocampal CA3 pyramidal cells (CA3-PCs). However, the cellular mechanism underlying this heterosynaptic modulation remains elusive. We found that high frequency MF inputs downregulate Kv1.2 in the CA3-PCs, and that the downregulation of Kv1.2 results in specific enhancement of PP-EPSPs. The NEURON simulation based on the known ion channel distributions on apical dendrites of CA3-PCs suggests that a concerted action of passive normalization of synaptic inputs and polarized distributions of ionic channels underlie a preferential generation of dendritic Na^+-spikes at distal apical dendrites, where PP inputs arrive. Accordingly, 10 nM tetrodotoxin, which specifically suppresses dendritic Na^+-spikes, brought back the enhanced PP-EPSPs to the baseline level. These results indicate that activity-dependent downregulation of Kv1.2 in CA3-PCs mediates MF input-induced heterosynaptic LTP of direct cortical synaptic inputs through preferential generation of Na^+-spikes at distal apical dendrites of a CA3-PC.

(COI: No)

CS05-2

Activity-dependent Homeostatic Plasticity of Hippocampal Mossy Fiber-CA3 Circuit

Lee, Kea Joo (*Korea Brain Research Institute, Daegu, Korea*)

Network activity homeostatically alters synaptic efficacy to constrain neuronal output. However, it is unclear how such compensatory adaptations coexist with synaptic information storage, especially in established networks. Here, we demonstrate that in mature hippocampal neurons in vitro, network activity preferentially regulated excitatory synapses within the proximal dendrites of CA3 neurons. These homeostatic synapses exhibited morphological, functional, and molecular signatures of the specialized contacts between mossy fibers of dentate granule cells and thorny excrescences (TEs) of CA3 pyramidal neurons. In vivo TEs were also selectively and bidirectionally altered by chronic activity changes. TE formation required presynaptic synaptoporin and was suppressed by the activity-inducible kinase, Plk2. These results implicate the mossy fiber-TE synapse as an independently tunable gain control locus that permits efficacious homeostatic adjustment of mossy fiber-CA3 synapses, while preserving synaptic weights that may encode information elsewhere within the mature hippocampal circuit.

(COI: No)

CS05-3

Physiological approaches to study presynaptic motor proteins in vesicle reuse pathways

Mochida, Sumiko (*Dept Physiol, Tokyo Med Univ, Tokyo, Japan*)

Myosins II and VI are actin-based cytoskeletal motors that drive actin dynamics and membrane transport at brain synapses, however, the molecular mechanism linking variation in neural activity to synaptic vesicle (SV) resupply is unknown. We combined genetic knockdown and direct physiological measurement of synaptic transmission from paired superior cervical ganglion neurons to show that myosins IIB and VI work individually in vesicle reuse pathways, having distinct dependency and time constants with physiological action potentials (AP) frequency. Myosin VI resupplied the readily releasable pool (RRP) with slow kinetics independently of firing rates but acted quickly within 50 ms after AP. Under high frequency AP firing, myosin IIB resupplied the RRP with fast kinetics in a slower time window of 200 ms. Myosin IIB-mediated SV resupply follows dynamin-1-mediated endocytosis, while myosin VI-mediated SV resupply follows dynamin-3-mediated endocytosis. Collectively, our findings show how myosins work in appropriate vesicle reuse pathways associated with specific firing patterns. This work was supported by #25290025 grants-in-aid for Scientific Research B and for Exploratory Research.

(COI: No)

CS05-4

Cellular and molecular mechanisms of synaptic circuit pruning and plasticity

Watanabe, Masahiko (*Hokkaido Univ. Grad. Sch. Med., Sapporo, Japan*)

Initial synaptic circuits formed by genetic programs are stero-typed with redundant and overlapping connections. After birth, activation of postsynaptic neurons refines immature circuits into mature ones in an activity-dependent manner. In this process, two forms of synapse refinement, i.e., synapse pruning and circuit plasticity, occurs efficiently and simultaneously during the early postnatal period called critical period. In the synapse pruning, active synapses are strengthened, while less active ones are eliminated. By critical period plasticity, projection fields of active afferents expand at the expense of those of less active ones. Through these refinements, neural circuits acquire functional topography, in which early life history of individual animals and humans is reflected. We have so far studied cellular and molecular mechanisms of synaptic refinement using gene-manipulated mice. In the symposium, I will introduce that molecules involving activity-dependent intracellular Ca^{2+} dynamics regulate the synapse pruning, such as NMDA receptors for barrel formation in the somatosensory cortex and P/Q-type Ca^{2+} channels and mGluR1 for climbing fiber and parallel fiber elimination in cerebellar Purkinje cells. On the other hand, glutamate transporters controlling extracellular glutamate concentrations magnify the circuit plasticity mediating the expansion of active circuits and reciprocal shrinkage of inactive ones.

(COI: No)

Committee Symposium 6

Recent Development of Physical Therapy Research on Motor Control

(March 22, 16:00～17:30, Room D)

CS06-1

Posture-movements and cognition of bodily information

Takakusaki, Kaoru (*Ctr Brain Funct & Med Eng, Asahikawa Med Univ.*)

Are we aware of the existence of our body and the motion of every part of the body during movements? A "loss of the awareness or the knowledge of the body" would deprive not only capability to achieve adaptive movements but also willingness and orientation. Real-time sensory signals during movements always inscribe our body information into the brain through visual, auditory, somatosensory and vestibular pathways. Sensory signals acting on the brainstem and the limbic system call "attention" and alter "emotional state", respectively. In addition those acting on cerebral cortex produce "body schema". The information (attention, emotional state and body schema) of the body is always updated like ever-changing scenes. The information provides us orientation and knowledge of embodiments that can be utilized for motor programing that occurs at the supplementary motor area (SMA) and premotor area (PM) as initial and restraint conditions. Body schema, which is constructed in the temporal and parietal association cortices, can be particularly essential in this process. The motor program includes that for volitional-guided precise movements and that for postural control of whole body that precedes the movement onset. Possibly, the former is achieved by the corticospinal projection and the latter can be accomplished by the cortico-reticular and the reticulospinal projections.

(COI: No)

CS06-2

Implicit adjustment of postural control strategy with a real-time feedback movable footplate

Kawashima, Noritaka (*Research Inst., NRCD, Saitama, Japan*)

Postural control relies on multisensory processing and its interaction to automatic control system which dominantly involves quick-responded reflex and vestibular system. Such control system enables us to maintain seemingly-unstable bipedal posture without special attention. Additional cortical demand would be increased when one faced to uncertain surroundings or unstable ground surface. We recently developed a real-time feedback movable footplate in order to get a better understanding of human postural control and to find a novel approach for the improvement of postural instability due to aging and disorders. In my talk, I will firstly show the results obtained from healthy subjects, which aimed to clarify postural responses due to augmented/reduced postural sway realized by established real-time feedback system. The results clarify that the real-time feedback has a potential to modulate the interaction between spinal reflex excitability and cortical command during upright standing. Then, I will show the preliminary results obtained from patients in order to discuss potential advantages of our developed system for the improvement of postural disorder.

(COI: No)

CS06-3

Meso-limbic system as a meta-learning center for motor recovery after neuronal damage

Nishimura, Yukio (*Dept Develop Physiol, Nat Inst Physiol Sci, Okazaki, Japan*)

It is generally believed that depression impedes functional recovery after neuronal damage such as spinal-cord injury. The ventral striatum is generally considered to regulate motivation, and not to be involved in the direct control of movements. However, it was found the activity of the VSt increased in association with activation of the motor cortex during recovery from spinal cord injury . This fact suggests that the interaction between VSt and motor cortex plays an important role for the reorganization of motor circuits after the SCI. Nevertheless, the causal relationship of the VSt for the motor recovery remains unclear. Here we show that the VSt plays crucial role in more direct control of dexterous finger movements by modulating the oscillatory activity of the motor cortex during the early stage of the recovery course. We found that high frequency oscillatory activity (200-400 Hz) of the VSt became enhanced and causally influences the activity of sensorimotor cortices during early stage of recovery. Reversible inactivation of the VSt caused severe deficit of the finger dexterity and diminished high frequency oscillatory activity of sensorimotor cortices during early stage of recovery. Our results first demonstrate that the VSt up-regulates the activity of sensorimotor cortices and can work as a meta-learning center for the recovery of motor functions after the neuronal damage.

(COI: No)

CS06-4

Interaction between gastrocnemius muscle weakness and moderate running exercise on rat knee joint cartilage

Ozawa, Junya (*Fac.Rehab.Hiroshima Int Univ., Hiroshima, Japan*)

Objective: Therapeutic exercises are used for symptomatic relief in patients with osteoarthritis of the knee. However, the interaction between ankle muscle dysfunction and exercise of the knee joint structure has not been studied.
Design: Gastrocnemius muscle weakness was induced by intramuscular injection of botulinum toxin type A (BTX) in skeletally mature rats. Moderate treadmill running (12 m/min for 60 min) was applied for 6 weeks in rats with and without BTX. Untreated animals were used as controls. Kinematic features of the hindlimb during locomotion were investigated by 3D motion analysis. Serum biomarkers of cartilage metabolism were investigated by ELISA. Cartilage thickness and chondrocyte density in the tibial plateau of the knee joint were also calculated histometrically.
Results: The gastrocnemius muscles were severely atrophied by BTX injection. Gastrocnemius muscle dysfunction was confirmed by locomotion analysis as an increased maximal dorsiflexion angle during the stance phase. Biomarker analysis revealed that 6 weeks of moderate running exercise facilitated the anabolic response of type II collagen. However, running-induced anabolism was significantly counteracted by BTX injection. In addition, thinning of the cartilage layer and a reduction in the chondrocyte density was also found in the BTX-injected rat knee after running for 6 weeks.
Conclusions: Exercise is proposed to have a positive effect on joint homeostasis. However, ankle muscle weakness may alter the mechanical environment of the knee and impair the integrity of joint cartilage with moderate exercise.
(COI: No)

CS06-5

Stimulation of functional recovery via the mechanisms of neurorepair by S-nitrosoglutathione and motor exercise following focal cerebral infarction in rats

Sakakima, Harutoshi; Matsuda, Fumiyo; Yoshida, Yoshihiro (*Sch. Health Sci. Med. Kagoshima Univ. Kagoshima, Japan*)

Ischemic stroke is major cause of neurological disability and big burden on the family and society. Physical exercise and pharmacological agents following stroke induce neurophysiological and neuroanatomical plasticity, leading to the recovery of function. In this session, we talk about the protective effects of physical exercise on neurovascular unit, including neurons, astrocytes, pericytes and extracellular matrix. Furthermore, we talk whether neurovascular protective agent, S-nitrosoglutathione (GSNO) in combination with motor exercise exerts a synergistic effect in stimulating the mechanisms of neurorepair, leading to accelerated and enhanced functional recovery following stroke. GSNO invokes anti-inflammatory effects on post-injury events mainly through the down regulation of the expression of NF-kB, adhesion molecules, cytokines, and inducible nitric oxide synthase. Stroke was induced by middle cerebral artery occlusion and reperfusion in adult male rats. Endurance exercise training reduced infarct volume, alleviated neurological deficits, enhanced expression of neurotrophic factor, promoted angiogenesis, and decreased apoptosis cell death. Combination of GSNO and exercise showed reduced infarction, decreased neuronal cell death, enhanced neurotrophic factors, and improved neurobehavioral functions. The protective effect of GSNO and exercise was blocked by the inhibition of Akt activity. GSNO and exercise aid functional recovery by stimulating neurorepair mechanisms.
(COI: No)

Committee Symposium 7

Neural mechanisms of acupuncture analgesia

(March 22, 17:30～19:00, Room D)

CS07-1

Recent advances in anatomical studies on the descending pain control systems

Senba, Emiko (*Osaka Yukioka College of Health Science, Osaka, Japan*)

Clinically relevant long-term pain relieving effects of acupuncture (AP) can be seen in a proportion of patients with chronic pain, such as chronic low-back pain. However, the mechanisms behind such effects are still obscure. It has been demonstrated that electro-AP-induced analgesia in experimental animals was antagonized by naloxone or methysergide, indicating the involvement of opioid peptides and serotonin (5-HT), i.e. descending pain inhibitory system. Stress-induced analgesia (SIA) and the activation of diffuse noxious inhibitory control (DNIC) may also contribute to the AP-induced analgesia. It has been demonstrated that the rostral ventromedial medulla (RVM) plays key roles in endogenous pain control system and AP can activate RVM neurons, thus inducing analgesia.Therefore, in this symposium, I'll focus on the detailed anatomy of the descending inhibitory system. Neurons in the RVM are divided into ON-, OFF- and neutral cells. 5-HT neurons belong to neutral cells. About 50% of RVM projection neurons, that project to spinal dorsal horn, are serotonergic. GABA containing RVM neurons have been considered to be local interneurons, but about 40% of RVM projection neurons were shown to be GABAergic. GABA synthesis in the RVM and superficial dorsal horn is a critical component in the descending inhibition. Recently it has been demonstrated that GABA synthesis in these neurons is suppressed in animals suffering chronic inflammatory and neuropathic pain. This suppression seems to be mediated by epigenetic regulation of GAD transcription. We have recently demonstrated that physical exercise can prevent this down-regulation.
(COI: No)

CS07-2

Mechanisms underlying descending modulation of spinal and medullary nociceptive neurons

Iwata, Koichi; Katagiri, Ayano; Shinoda, Masamichi (*Dept. Physiol, Sch Dent, Nihon Univ, Tokyo, Japan*)

It is well known that peripheral nociceptive inputs come up to the spinal and medullary dorsal horn, and those are sent to the higher central nervous system via medial and lateral ascending pathways. The activities of nociceptive neurons in these ascending pathways are modulated by the descending modulation system. Descending inhibitory system is well known to be involved in inhibition of responses of spinal and medullary nociceptive neurons via descending serotoninergic or noradrenergic system. Rostral Ventral Medulla (RVM) is known to be a key nucleus involving in descending modulation of nociceptive neurons. Furthermore, activities of nociceptive neurons are enhanced via RVM neurons associated with peripheral nerve injury or inflammation. There are 3 groups of neurons involving in modulation of the nociceptive neurons in the RVM, ON cells, OFF cells and neutral cells. ON cells are involved in the enhancement of nociceptive neurons, OFF cells are inhibition and neutral cells are not involved in modulation of nociceptive neurons. Though these 3 types of RVM neurons are thought to be involved in modulation of spinal and medullary nociceptive neurons, detail mechanisms underlying descending modulation of nociceptive neurons are not fully understood. In this symposium, known mechanisms underlying descending modulation system via RVM will be presented, and the modulation of nociceptive neurons under pathological conditions will be discussed.
(COI: No)

CS07-3

Chronic stress and pain

Kiyama, Hiroshi (*Grad.Sch.Med. Nagoya Univ., Nagoya, Japan*)

Patients suffering from cryptogenic symptoms, such as chronic fatigue syndrome (CFS) and fibromyalgia syndrome (FMS), display chronic widespread pain (hyperalgesia and/or allodynia) and multiple symptoms, including severe fatigue, sleep disturbance, malaise and cognitive dysfunction. Among these symptoms the abnormal pain sensation could be the most serious, however its pathophysiology remains unknown. We used a multiple continuous stress (CS) model in rat, which were housed in a cage with a low level of water (1.5 cm in depth) for 5 days. Using the von Frey and Randall Seritto tests, the model rat showed the mechanical allodynia at plantar skin and mechanical hyperalgesia at the anterior tibialis (i.e. muscle pain). Although no signs of inflammation and injury incidents were observed in both the plantar skin and leg muscles, microglial accumulation and activation were observed in L4-L6 dorsal horn of CS rats. To evaluate an implication of microglia in pain, minocycline was intrathecally administrated. Minocycline significantly attenuated CS-induced mechanical hyperalgesia and allodynia. Although the mechanism underlying the local activation of microglia remains obscure, these results indicated that activated microglia were involved in the development of abnormal pain in CS animals, suggesting that the pain observed in CFS and FMS patients may be partly caused by a mechanism in which microglial activation is involved.
(COI: No)

CS07-4

What is the biological significance of emotion in pain and its regulation?

Kato, Fusao (*Dept Neurosci, Jikei Univ Sch Med*)

According to the definition by Charles Darwin (1872), emotion is automatic responses to events in an organism's environment that help it to survive. Primordial sensory sensations, such as taste, olfaction and nociception, are often the detectors of aversive, potentially harmful events closely related with survival, and thus with the emotion. In addition to the neural connections that allow avoiding on-going harmful situations (withdrawal reflex, e.g.), the suffering function of the pain is thought to have evolved so that it enables the organism to alter its behavioral program through neural plasticity after the aversive experience. The capsular part of the central nucleus of the amygdala receives direct nociceptive information from the spinal cord via the spino-parabrachio-amygdaloid (SPA) pathway, in addition to the indirect thalamocortical pathway. This SPA pathway has been identified to be essential in the nociception-emotion link and its plasticity. Unlike acute pain that has pro-survival beneficial functions, the chronic pain, characterized by its potent suffering aspect, is pathological and of no biological significance. In human patients, chronic pain is strongly linked with aberrantly elevated spontaneous activities in brain areas involved in emotion, such as the amygdala. In animal models of chronic pain, a robust synaptic potentiation has been described in the amygdala. On the basis of our electrophysiological and functional MRI studies in rodent models of chronic pain, we argue that the amygdala would be an important target of the chronic pain therapies.

(COI: No)

CS07-5

Neural mechanisms of anti-nociceptive effect induced by gentle skin stimulation

Hotta, Harumi (*Dept Auton Neurosci, Tokyo Metropol Inst Gerontol, Tokyo, Japan*)

Somato-sympathetic reflexes induced by noxious stimuli are involved in pathogenesis of chronic pain, therefore their managements are of clinical importance. In anesthetized rats, skin touch can suppress somato-cardiac sympathetic C reflex induced by excitation of unmyelinated C-afferent fibers of the plantar nerve. The effect was dependent on texture in contact with skin: a disc having orderly arranged microcones (similar to a fingertip), but not a flat disk, was effective. Similarly, in conscious humans, microcones, but not flat disc, suppressed noxious heat-induced somato-cardiovascular responses. To clarify further mechanisms, we compared effects between touch with and without microcones. Tactile perception or glucose metabolism in the somatosensory cortices (measured by positron emission tomography) was not different between two different touch. However, the metabolism of anterior cingulate cortex was higher during touch with microcones. Among three different types of low-threshold mechanoreceptive fibers of skin, $A\delta$;- and C-fibers, but not $A\beta$-fibers, showed greater excitations during touch with microcones. The inhibitory effect of somato-cardiovascular reflexes by microcone was abolished following intrathecal application of naloxone into the lumber spinal cord. We suggest that excitation of low-threshold $A\delta$ and C afferents releases spinal opioids, resulting in the inhibition of nociceptive transmission into the sympathetic nerves. Such spinal mechanisms apart from cognition may help to explain relief of chronic pain by gentle somatic stimulation, such as Japanese acupuncture.

(COI: No)

CS07-6

Role of anterior cingulate cortex in descending antinociceptive effects produced by acupuncture stimulation

Toda, Kazuo (*Integr Sensory Physiol, Nagasaki Univ, Nagasaki, Japan*)

It is well-known that descending inhibitory mechanisms are strongly involved in acupuncture analgesia.This descending inhibition is the most powerful analgesic mechanism in the CNS. Generally, descending pathways project to the spinal cord from various pain suppression centers as revealed by neuroanatomical studies.These centers include the periaqueductal gray matter (PAG), nucleus raphe magnus (NRM) and other areas in the ventromedial medulla. On the other hand, it is recently reported that the anterior cingulate cortex (ACCX) is involved in emotional pain perception and modulating pain sensation. Indeed, nociceptive responses can be recorded non-somatotopically in the rat and these responses are well modulated under physiological disturbance, such as, stress. Anatomical studies also indicate that there are dense descending projections from the ACCX to the PAG. Because the PAG is a key link in the descending analgesic system projecting to the spinal cord through the NRM, it can be assumed that the ACCX is concerned with the control of the descending analgesic system activated by acupuncture stimulation. However, there are no available data concerning the response properties and the role of the ACCX neurons following acupuncture stimulation. The present study shows that acupuncture stimulation can predominantly provoke inhibitory effects on the spontaneous activities of descending ACCX neurons. Since it is reported that a majority of the functional connection between ACCX and PAG is inhibitory, disinhibition of the PAG is closely related to produce descending acupuncture analgesia.

(COI: No)

Committee Symposium 8

Japan-Germany Joint Symposium

New bridge between Germany and Japan for basic medical sciences

(March 23, 9:00～10:30, Room E)

CS08-1

Anatomische Gesellschaft and Japanese Association of Anatomists (JAA): a long lasting relationship between anatomists of both societies: past, present and future

Paulsen, Friedrich P. (*Department of Anatomy II, Friedrich Alexander University Erlangen-Nuremberg*)

In 1870, the new Japanese Government had decided the implementation of the German Medical curriculum. Thus, many Japanese studied medicine in Germany or worked as medical doctors in Germany. This was interrupted by World War II, whereupon both countries were faced with severe destruction. Nevertheless, based on the old connections, Japanese students started coming back to Germany comparatively soon thereafter. Here, the German Departments of Anatomy played a crucial role. The talk will describe some of these old connections and will highlight successful collaborations between Japanese and German Anatomists such as those between Yutaka Sano and Wolfgang Bargmann or between Chihiro Yokochi and Johannes Rohen. The Chihiro and Kiyoko Yokochi Fund that is open to young German anatomists, but still needs collaborations with Departments of Anatomy in Japan, will be introduced and explained as an easy option for exchange of young researchers from Germany with Departments of Anatomy in Japan. Moreover, the talk will give an overview about research areas and current themes in Departments of Anatomy across Germany to provide JAA members with an idea of how anatomy is currently organized and working in Germany. Finally, a major intention of the talk will be to present the Anatomische Gesellschaft and its members as an attractive partner for JAA, and to stimulate, once again, a deeper connection between anatomists of both societies.

(COI: No)

CS08-2

My teachers in Japan and Germany -glycine oder das Glyzin-

Sato, Kohji (*Sch.Med.Hamamatsu Univ., Shizuoka, Japan*)

Here, I want to present the audience my three teachers in Japan and Germany. The first teacher is Prof. Keiya Tada. He was a former professor of the department of pediatrics, Tohoku Univ.. He devoted his life to studying congenital metabolic disorders, especially, hyper-glycinemia, which is manifested by very high concentrations of glycine in blood, etc and very sever symptoms including seizures. He found that the glycine cleavage enzyme is defected in the patients. As this disease has very severer neurological symptoms compared with other metabolic disorders, he asked me to investigate where the glycine cleavage enzyme is in the CNS. To do this, I went to the departmemt of Anatomy in Osaka Univ.. There, I met the second teacher, Prof. Masaya Tohyama. He is a specialist of neuroanatomy. I learned immunohistochemical technique and found that the glycine cleavage enzyme is specifically expressed in astorcytes. After that, I learned in situ hybridization technique and investigated the distributions of glycine receptors. From that point, I decided to deeply investigate the glycinergic system. After getting PhD degree in Osaka Univ., I joined the department of neurochemistry, Max-Planck institute, Frankfurt. There I met the third teacher, Prof. Heinrich Betz, who had cloned many glycine receptors. In his laboratory, I studied glycine transporter regulation via phosphorylation. Through above-mentioned stories, I want to show the audience what the glycinergic system is and my sweet memories with the teachers in Japan and Germany.

(COI: No)

CS08-3

Membrane biophysics: Quantitative understanding of neural signaling

Sakaba, Takeshi (*Grad Sch Brain Science, Doshisha Univ*)

In the past 15 years, we have examined the mechanism of transmitter release at the large nerve-terminal called the calyx of Held located in the brainstem, Ca-uncaging method, which has been introduced first by Almers, Neher and Zucker and others for studying Ca-secretion coupling in secretory cells, has been used for determining the Ca-dependence of neurotransmitter release and synaptic vesicle replenishment at the large central synapse. More recently, in collaboration with German scientists, we extended the study using super-resolution microscopy (STED), molecular genetics (the use of KO and Ki mice) and biochemistry, to understand the molecular mechanisms of neurotransmitter release. Also, in order to compare the properties among different types of synapses, we are currently running the collaborative program among Japanese and European synaptic physiologists. Such an effort is important for obtaining the basic principle of synaptic transmission in a quantitative manner.The study was supported by Core-to-Core Program A. Advanced Research Networks.

(COI: No)

Committee Symposium 9

Japan-China Joint Symposium - Towards FAOPS2019

Recent Advances in Organellar Morphology and Physiology

(March 23, 13:30～15:00, Room E)

CS09-1

Polar body genome transfer for preventing the transmission of inherited mitochondrial diseases

Sha, Hongying; Wang, Tian; Ji, Dongmei; Zhang, Helen L; Chen, Dawei; Cao, Yunxia; Zhu, Jainhong (*Dept Neurobiol, Inst Brain Sci, Sch Basic Med Sci, Fudan Univ, Shanghai, China*)

Inherited mitochondrial DNA (mtDNA) diseases transmit maternally and cause severe phenotypes. Since no effective treatment is available, nuclear genome transfer between patients' and healthy eggs to replace mutant mtDNAs holds promises. Since polar body contains very few mitochondria and share same genomic scale as oocyte, here we perform polar body transfer to prevent the transmission of inherited mtDNA variants. We compare the value of different germline genome transfer, i.e., spindle-chromosome transfer (ST), first polar body transfer (PB1T), pronuclear transfer (PNT), and second polar body transfer (PB2T), to exchange mtDNA genotype in a mouse model. Reconstructed embryos support normal fertilization and produce live offspring. Strikingly, genetic analysis confirms polar body generated-offspring possesses minimal donor mtDNA carry-over compared with spindle-chromosome (low/medium carry-over) and pronuclear (medium/high carry-over) transfer. All PB1T offspring contains undetectable mtDNA heteroplasmy level (0%), which is significantly lower than ST offspring (5.53% ± 1.43%). PB2T infants possessed 1.7% ± 2.8% mtDNA carry-over on average, which is significantly lower than PNT infants (23.7% ± 11.1%). Importantly, all F2 PB1T progeny still harbors undetectable heteroplasmy level. Our preclinical model demonstrates polar body transfer, especially PB1 transfer, which circumvent the possibility of mtDNA heteroplasmy in offspring, holds great potential in preventing the transmission of inherited mtDNA diseases.

(COI: No)

CS09-2

Mitochondrial DNA damage and disease

Zhang, Xiuying; Zhou, Deshan (*Cap.Med.Univ.Sch.Med., Beijing, China*)

Human mtDNA is a 16, 569 bp, plasmid-like circular DNA molecule that encodes 13 gene products required for electron transport and oxidative phosphorylation. Increased ROS production by mitochondria is likely to damage mtDNA and impair mitochondrial function leading to their inability to completely reduce molecular oxygen and further injury to the mitochondria. The lack of protective histone and reduced fidelity of DNA replication and abundant DNA repair mechanisms make mitochondrial genome more sensitive to the attacks of free radicals produced in mitochondria, or other DNA damaging agents. Mitochondrial DNA (mtDNA) is 10 to 20 times more vulnerable to oxidative damage and subsequent mutations than nuclear DNA. Only base excision (BER) and perhaps single strand break repair (P-PARP) are functioning in mitochondria. Neil1 and OGG1 are two major repair enzymes for mtDNA. In the studies of alcoholic liver disease, we found that ethanol feeding in IL-6 KO mice induced significant mtDNA deletions that were associated with the loss of the mitochondrial membrane potential, greatly diminished levels of cytochrome c oxidase subunit-I synthesized in the mitochondria, along with diminished ATP and mtDNA repair enzymes levels. Thus oxidative injury that was fairly well tolerated in WT mice fed ethanol had highly deleterious effects in IL-6 KO mice. In these experiments we have shown that IL-6 is necessary to provide cell cycle checkpoints via induction of p21 and p53, and stimulate the transcription of DNA repair enzymes.

(COI: No)

CS09-3

Ion channels in perinuclear endoplasmic reticulum membrane

Maruyama, Yoshio (*Dept Cell Physiol, Grad Sch Med, Tohoku Univ, Sendai, Japan*)

Finishing *in situ* identification of ion channels in the cell membrane, patch-clampers intend the same in the organelle membranes including those in the endoplasmic reticulum (ER) or the nucleus. Then immediately we face to a critical problem; how one can say that our membrane preparation keeps its normal orientation without contamination of the plasma membrane, and is suited to every patch-clamp technique. We discuss it in the section of making preparation while showing its shape.

I demonstrate the presence of some ion channels in the peri-nuclear ER membrane in mammalian exocrine gland cells, mouse pancreatic acinar cells. I show that 1) Maxi-K^+ channels, its expression depending animal age, 2) anion-channels balancing the electroneutrality, 3) water-channels regulating compartment volume by osmosis, and 4) unknown other channels.

Applying capacitance measurements to the preparation, I show that a rise in the compartment Ca^{2+} increases membrane capacitance, an indicator of the membrane area. It suggests that the rise connects adjacent compartment together.

I propose a scenario of the peri-nuclear ER shape change (compartment change). As cell s up-growing proceeds, their ATP content increases. The Ca^{2+} pump in the ER (SERCA) functions and consequently leads to the increase in compartment Ca^{2+} concentration. Then it activates Maxi-K^+ channels and Cl channels, which together trigger the water osmosis. Meanwhile the compartment Ca^{2+} connects the neighbor compartment together. Thus, the area and volume of the ER extend for the protein formation and storage.

(COI: No)

CS09-4

Ultrastructural analyses of the formation of autophagic isolation membrane in mammalian cells

Waguri, Satoshi (*Fukushima Med. Univ. Sch. Med., Fukushima, Japan*)

Recent findings have suggested that autophagic isolation membrane (IM) originates from a domain of endoplasmic reticulum (ER) called "omegasome". However, its fine structure and detailed positional relationships to the ER and IM during autophagosome formation remain unclear. In the present study, we used *Atg3*-deficient mouse embryonic fibroblasts (MEFs) expressing a marker of omegasome, GFP-tagged double FYVE domain-containing protein 1 (GFP-DFCP1), and found that GFP-DFCP1 was localized on tubular or vesicular elements adjacent to the IM rims by correlative light and electron microscopy and immuno-electron microscopy. Moreover, we developed a fixation protocol for electron microscopy (EM) using a mixture of paraformaldehyde, glutaraldehyde, and osmium tetroxide as a primary fixative, for clear-cut detection of IM and associated vesicular or tubular structures. By EM analyses including serial ultra-thin sections and electron tomography, we observed a cluster of thin tubular structures between the IM edges and ER, part of which were continuous with IM and/or ER. These IM-associated tubular structures (IMATs) were observed in several cell lines and MEFs deficient for *Atg5*, *Atg7*, or *Atg16L1*, but not in *FIP200*-deficient cells, suggesting that they are relevant to earlier events in autophagosome formation. Taken together, our findings indicate that IMATs represent a part of omegasome during completion steps of autophagosome formation.

(COI: No)

Committee Symposium 10

Future prospect of anatomical, pharmacological, and physiological journals

(March 23, 13:30～15:00, Room J)

CS10-1 JPhysS's way to go as an international scientific journal in Physiology

Ishikawa, Yoshihiro CVRI, Yokohama City Univ Sch Med, Yokohama, Japan

CS10-2 Anatomical Science International: past, present and future

Yorifuji, Hiroshi Grad. Sch. Med. Gunma Univ., Maebashi, Japan

CS10-3 New Platform from International Pharmacological Sciences

Fukunaga, Kohji Dept Pharm, Grad Sch Pharm Scis, Tohoku Univ, Japan

Award Presentations（Oral）

MD Scientist Program（Oral）

Hiroshi and Aya Irisawa Memorial Award Symposium

Brain-gut association via peptides and amines

(March 22, 17:30～19:00, Room A)

IS-1

Effects of food deprivation on the hypothalamic feeding-regulating peptides gene expressions in serotonin depleted rats

Yoshimura, Mitsuhiro[1]; Hagimoto, Marina[1]; Matsuura, Takanori[1]; Ohkubo, Junichi[1]; Ohno, Motoko[1]; Maruyama, Takashi[1]; Ishikura, Toru[1]; Hashimoto, Hirofumi[1]; Kakuma, Tetsuya[2]; Yoshimatsu, Hironobu[2]; Terawaki, Kiyoshi[3]; Uezono, Yasuhito[3]; Toyohira, Yumiko[4]; Yanagihara, Nobuyuki[4]; Ueta, Yoichi[1] (*[1]Dept Physiol, Sch Med, UOEH, Kitakyushu, Japan; [2]Dept Internal Medicine 1, Sch Medicine, Oita Univ, Oita, Japan; [3]Div Cancer Pathophysiol, Gr Dev Mol diag and Ind Ther, Natl Cancer Cent Res Ins, Tokyo, Japan; [4]Dept Pharmacol, Sch Med, UOEH, Kitakyushu, Japan*)

We examined the effects of serotonin (5-HT) depletion induced by peripheral injection of 5-HT synthesis inhibitor p-chlorophenylalanine (PCPA) on the expression of feeding-regulating peptides expressions by using in situ hybridization histochemistry in adult male Wistar rats. PCPA pretreatment had no significant effect on basal levels of *oxytocin*, *corticotropin-releasing hormone* (*CRH*), *thyrotropin-releasing hormone* (*TRH*), *pro-opiomelanocortin* (*POMC*), *cocaine and amphetamine-regulated transcript* (*CART*), *neuropeptide-Y* (*NPY*), *agouti-related protein* (*AgRP*), *melanin-concentrating hormone* (*MCH*) or *orexin* in the hypothalamus. 48 h food deprivation caused a significant decrease in *CRH*, *TRH*, *POMC*, and *CART*, a significant increase in *NPY*, *AgRP* and *MCH*. After PCPA treatment, *POMC* and *CART* did not decrease despite food deprivation. *NPY* significantly increased by food deprivation with PCPA, but was attenuated compared to food deprivation without PCPA. These results suggest that the serotonergic system in the hypothalamus may be involved in the gene expression of *POMC*, *CART*, and *NPY* related to feeding behavior.

(COI: No)

IS-2

Roles of peptides and amines in the regulation of the colorectal motility via the spinal cord

Shiina, Takahiko[1]; Naitou, Kiyotada[1]; Nakamori, Hiroyuki[1]; Sano, Yuuki[1]; Ikeda, Azusa[1]; Hirayama, Haruko[2]; Shimizu, Yasutake[1] (*[1]Dept Basic Vet Sci, Lab Physiol, Unit Grad Sch Vet Sci, Gifu Univ, Gifu, Japan; [2]Dept. Animal Resources, Okayama Univ., Okayama, Japan*)

Colorectal motility is regulated by the enteric neurons locally and the defecation center systemically. The lumbosacral defecation center regulates colorectal motility via sympathetic lumbar colonic nerve and parasympathetic pelvic nerve. On the other hand, the brain stem defecation center modulates the lumbosacral defecation center through descending spinal neural pathway and then affects colorectal motor activity. Although it is speculated that various neurotransmitters including peptides and amines are involved in the regulation of colorectal motility by the defecation center, precise mechanisms remain to be clarified. Therefore, we have investigated regulatory mechanisms of colorectal motility by the lumbosacral defecation center. For assessing colorectal motility, we have used in vivo recording system. In brief, animals were anesthetized and their distal colon and anus were cannulated to measure the intracolorectal pressure and propelled intraluminal liquid volume. We have demonstrated that intrathecal injection (L6-S1 region of the spinal cord) of ghrelin enhances colorectal motility through activation of the lumbosacral defecation center. In addition, we found that several amines acting on the spinal cord enhance colorectal motility. In this presentation, we will summarize roles of peptides and amines in the regulation of the colorectal motility via the spinal cord.

(COI: No)

IS-3

5-HT$_4$ receptor-mediated facilitation of neurogenesis of enteric neurons from transplanted brain-derived neural stem cells

Takaki, Miyako (*Dept Mol Pathol, Sch Med, Nara Med Univ, Kashihara, Japan*)

Two photon-excited fluorescence microscopy (2PM), can provide deeper optical penetration (several hundred μm) in in vivo preparations. We have used this approach in Thy1-promoter YFP mouse after gut transection and anastomosis. The fetal brain-derived neural stem cells (NSC) were transplanted from the tail vein after treatment with red fluorescent cell linker, PKH26. We obtained clear three-dimensional imaging of newborn enteric neurons generated from enteric neural progenitors (mobilized resident NSC; green fluorescence) and those from transplanted NSC (red fluorescence). Neurogenesis was promoted by oral application of a 5-HT$_4$-receptor agonist, mosapride citrate (MOS: 100 μM) and this promotion was inhibited by simultaneous application of a 5-HT$_4$- receptor antagonist, SB-207266 (50 μM), indicating 5-HT4 receptor-mediated facilitation of neurogenesis. Number of new neurons from the transplanted NSC was much smaller (approximately 10%) than that from the mobilized resident NSC, but the facilitating effect of MOS was similar between the transplanted and resident NSC. The distribution pattern of new neurons from the transplanted NSC was similar to that of new neurons from the resident NSC. After in vivo imaging, PGP9.5 (+) cells (neurons), and PKH26 (+) and YFP (+) cells were compared by confocal microscope. New enteric neurons overlapped with PKH26 (+) or YFP (+) cells in the deep tissue of mouse small intestine.

(COI: No)

The Winning Lectures of Encouragement Award of the JAA

(March 22, 16:00～17:30, Room A)

AS-1 Functional organization of the neural circuit in the inferior colliculus

Ito, Tetsufumi — Division of Anatomy and Neuroscience, Faculty of Medical Sciences, University of Fukui

AS-2 Molecular imaging analysis of small GTPases in the regulation of macropinocytosis and phagocytosis

Egami, Youhei — Department of Histology and Cell Biology, School of Medicine, Kagawa University

AS-3 Application of cadavars embalmed by the saturated salt solution method for surgical training

Hayashi, Shogo — Department of Anatomy, Tokyo Medical University

AS-4 Molecular and anatomical evidence for input pathway- and target cell type-dependent regulation of glutamatergic synapses

Yamazaki, Miwako — Department of Anatomy, Graduate School of Medicine, Hokkaido University

MD Scientist Training Program

(March 21, 15:30～18:00, Room A)

MD-S1

Oncogenic Notch1 activities in esophageal squamous cell carcinoma

Otsuka, Yuki[1,2]; Tanaka, Koji[2]; Whelan, Kelly[2]; Kinugasa, Hideaki[3]; Rhoades, Ben[2]; Ikeda, Fusao[3]; Yamamoto, Kazuhide[3]; Nakagawa, Hiroshi[2] (*[1]Med.Sch.Okayama Univ., Okayama, Japan; [2]GI division, Univ.of Pennsylvania, Philadelphia, USA; [3]Dept. Gastroenterol & Hepatol, Okayama Univ., Okayama, Japan.*)

Introduction: Notch signaling regulates stem cells and differentiation; however, its role in tumor biology remain elusive. We investigated the functional consequences of Notch activation in esophageal squamous cell carcinoma (ESCC).
Methods: Immunohistochemistry was performed using primary tumors from ESCC patients and a 4-Nitroquinoline 1-oxide-induced mouse model of ESCC as well as xenograft tumors. ICN1, the active form of Notch1, was ectopically expressed in ESCC cells (TE11) in a tetracycline-inducible fashion to determine how ICN1 affects sphere formation (self-renewal), anchorage-independent growth in soft agar, and tumor growth in immunodeficient mice.
Results: ICN1 was expressed at the invasive tumor fronts in primary ESCC and was associated with poor prognosis in patients. Immunohistochemistry suggested epithelial-mesenchymal transition in murine ESCC cells expressing ICN1. In TE11 cells, ICN1 stimulated sphere formation and increased colony size and number in soft agar. In xenograft tumors, ICN1 promoted tumor growth with increased cell proliferation and decreased squamous-cell differentiation.
Conclusions: Our data suggest that Notch1 may be activated in invasive ESCC cells with increased malignant properties. Notch signaling may have oncogenic activities in the pathogenesis of ESCC.

(COI: No)

MD-S2

Analysis of Arhgef2 phosphorylation at Ser885

Oda, Kaishu (*Nagoya Univ., Nagoya, Japan*)

abstractRhoA is one of the small GTP-binding proteins (small G proteins) which mediates F-actin polymerization and regulates cytoskeleton and cell adhesion. The small G proteins function as molecular switches, cycling between inactive GDP-bound state and active GTP-bound state. Arhgef2 (Lfc or GEF-H1) is a major Rho guanine exchange factor (RhoGEF), that activates RhoA by exchanging GDP for GTP. Depending on PKA, phosphorylation at Ser885 of Arhgef2 inactivates its GEF activity. In vitro investigation of the Ser885 phosphorylation has been conducted, however, the effect of such phosphorylation in vivo yet remains unknown. Assuming that phosphorylation of Arhgef2 at Ser885 alters cell morphology, we tried to produce Ala885 mutant Arhgef2 mouse with CRISPR/Cas9 system targeting Arhgef2 Ser885. We checked F0 mice and have detected knock-in allele in some mice. In the future, we will examine RhoA activity, cell morphology and behavior in knock-in mice in order to analyze the functions of Arhgef2 phosphorylation at Ser885.

(COI: No)

MD-S3

The remodeling of somatotopic area in intact hemisphere was improved by application of dehydroepiandrosterone after focal infarction in somatosensory cortex

Obi, Kisho; Takatsuru, Yuusuke; Amano, Izuki; Koibuchi, Noriyuki (*Dept. Integrative Physiol., Gunma Univ. Grad. Sch. Med, Gunma, Japan*)

The contralateral hemisphere of infarction play an important roles for functional compensation in somatosensory cortex (SSC) after focal ischemia (Takatsuru et al., J.Neurosci., 2009). Recently, it is reported that infarction of the unilateral SSC changes the somatotopic area in the intact contralateral SSC (Takatsuru et al., J.Neurosci., 2013). In this study, we examined the changes of somatotopic area in contralateral (left) SSC during recovery phase by using electrophysiological technique. Sensory responses from the right paw are innately process in the left SSC. When the right SSC is infarcted, sensory responses from left paw became also processed in the left SSC. However, the size of the receptive field which responded from right and left limb somatosensory stimulation in 4 weeks (wks) after stroke significantly increased compared with those in 2wks after the stroke. This change was improved by repeated application of dehydroepiandrosterone (DHEA), which is demonstrated to have a neuroprotective effects, during 2 to 3wks after stroke. We next aim to clarify the underlying mechanisms by using electrophysiological and molecular biological techniques. Previously reported that DHEA is metabolized to estradiol in a brain (Elkihel et al., Steroids., 2012). Thus, next we will measure the amount of estradiol in a brain by ELISA and determine the expression of estradiol receptor by Western blotting.

(COI: No)

MD-S4

Identities of FoxP2-positive retinal ganglion cells in the visual system of ferrets and mice

Sato, Chihiro[1,2,3]; Ebisu, Haruka[2,3]; Ichikawa, Yoshie[2,3]; Kawasaki, Hiroshi[2,3] (*[1]Fac Sch Med, Univ of Tokyo, Tokyo, Japan; [2]Dept Biophys Genet, Grad Sch Med Sci, Kanazawa Univ, Ishikawa, Japan; [3]Brain/Liver Interface Med Res Ctr, Kanazawa Univ, Ishikawa, Japan*)

The mammalian retina contains principally two subtypes of retinal ganglion cells (RGCs). Magnocellular RGCs (M-RGCs) send visual information about object motion to M cells in the LGN in the thalamus, and parvocellular RGCs (P-RGCs) transmit information about form discrimination and color recognition to the lateral geniculate nucleus (LGN) along by parallel visual pathways. These RGC subtypes and parallel pathways are believed to be a fundamental basis of visual recognition. However, because the RGC subtypes are not well-developed in mice, the molecular properties of these RGC subtypes are still unknown. Using the ferret, which has M cells and P cells, we recently uncovered that FoxP2 is selectively expressed in P cells in the LGN of ferrets and monkeys (Iwai et al., 2013). This previously study led us to hypothesize that FoxP2 is selectively expressed in P cells also in the retina. We examined FoxP2 expression patterns in the ferret retina and found that FoxP2 was expressed in a small subset of RGCs and amacrine cells. Our morphological analyses suggested that FoxP2-positive RGCs were X cells (P-RGCs in ferrets). Interestingly, as in the case of ferrets, Foxp2 was also expressed in a small subset of RGCs in mice. We are currently examining the role of Foxp2 in the development of RGC identities using mice. Our results should be helpful for understanding the molecular mechanisms underlying RGC development. COI:NO

(COI: No)

MD-S5

Identification of novel interacting partner for SHP-1

Sugimoto, Ayano; Fujita, Yuki; Kimura, Yuriko; Yamashita, Toshihide (*Dept.Mol. Neurosci.Grad.Sch.Med.Osaka Univ.Osaka, Japan*)

Once the adult central nervous system (CNS) is damaged, it is difficult to regenerate. Under the existence of myelin-derived inhibitors of axonal regeneration, it is known that paired immunoglobulin-like receptor B (PIR-B) binds to tropomyosin receptor kinase (Trk), which enhances regeneration of the injured CNS. Then, Src homology 2-containing protein tyrosine phosphatase (SHP) is recruited to PIR-B, and inhibits axonal regeneration by dephosphorylation and inactivation of Trk receptor. Furthermore, under the circumstances of reduced expression of SHP-1 using small interfering RNA (siRNA), axon regeneration is enhanced in mice optic nerve after injury. Hence, it is suggested that SHP-1 may be an effective molecular target for CNS injury. However, as SHP-1 is expressed ubiquitously, inhibition of SHP presumably causes severe adverse event. Thus, we aimed to clarify the molecular mechanism of inhibition of axonal regeneration by SHP, and identify appropriate therapeutic target molecule.We adopted a mass spectrometry (MS)-based approach to identify proteins that associate with SHP-1. Among the SHP-1-associated proteins, we focused on microtubule-associated protein 1B (MAP1B) and filamin A (FLNA). MAP1B is abundantly expressed in neurons, and regulates the organization of microtubules in neurites. FLNA is a cytoskeleton protein that crosslinks actin filaments. We examined the physiological functions of the interactions between SHP-1 and these proteins.

(COI: No)

MD-S6

A novel protein in the central pair apparatus of *Chlamydomonas* flagella

Tani, Yuma; Yagi, Toshiki; Oda, Toshiyuki; Kikkawa, Masahide (*Grad. Sch. Med., The Univ. of Tokyo, Tokyo, Japan*)

Eukaryotic cilia and flagella are highly intricate cell organelle that plays important roles in various organs as propellers or sensors. Therefore, impaired cilia/flagella in human causes various diseases, which are called ciliopathies. Motile cilia/flagella share a well-conserved "9+2" structure, which consists of nine doublet and two central pair microtubules. In this structure, the central pair apparatus is considered as a mechanical regulator, which turns on or off the activity of dynein arms on the doublet microtubules via radial spokes. However, the functions or components of central pair apparatus are not fully understood.

In this study, we identified a new flagellar-associated protein (FAP) as a component of central pair apparatus of *Chlamydomonas reinhardtii* flagellar axoneme. From an analysis of FAP-deficient mutants, we showed that the deficiency of FAP in flagellar axonemes reduces the flagellar motility and affects the bridge structure between the two microtubules in the central pair apparatus. These results suggest that FAP is a component of C1-C2 bridge structure in *Chlamydomonas* central pair apparatus and the structural defect by its deficiency affects the flagellar motility.

(COI: No)

MD-S7

Systems pharmacology of teratogenic action by valproic acid

Murakami, Soichiro[1]; Nishimura, Yuhei[1,2,3,4,5]; Sasagawa, Shota[1]; Ashikawa, Yoshihumi[1]; Kawabata, Miko[1]; Cho, Beibei[1]; Umemoto, Noriko[1,2,3,4,5]; Tanaka, Toshio[1,2,3,4,5] ([1]*Grad. Sch. of Med, Mie Univ, Japan;* [2]*Systems Pharmacology, Grad. Sch. of Med, Mie Univ, Japan;* [3]*Medical Zebrafish Research Center, Mie Univ, Japan;* [4]*Omics Medicine, Industrial Technology Innovation Institute, Mie Univ, Japan;* [5]*Bioinfo, Life Sci. Res. Center, Mie Univ, Japan*)

It is well known that valproic acid (VPA) has a histone deacetylase inhibition activity and exposure to the chemical during developmental period causes fetal valproate syndrome. However, the detailed mechanism has been unknown. To elucidate the molecular mechanism, we exposed VPA to fertilized eggs of zebrafish and analyzed the gene expression variations comprehensively using DNA microarray. Besides, we compared the transcriptome data in Gene Expression Omnibus data repository. The microarray data included the gene expression variations in embryos of zebrafish or mouse, and mouse embryonic stem cell exposed to VPA. As a result, we discovered that the expression of epc2 was significantly decreased by VPA exposure. EPC2 is related to 2q23.1 microdeletion syndrome characterized by psychomotor retardation, seizure, and stereotypic repetitive behavior. Then, we knocked out epc2 gene in zebrafish using TALEN to analyze the functional role of epc2 downregulation by VPA exposure. We were able to demonstrate that the expression of several genes were similarly dysregulated between zebrafish exposed to VPA and epc2-KO zebrafish, suggesting that epc2 may be involved at least partly in the neurotoxicity of VPA.

(COI: No)

MD-S8

A novel role of Regnase-1 in the iron homeostasis and anemia

Yoshinaga, Masanori; Takeuchi, Osamu (*Inst. Virus Res., Kyoto Univ., Kyoto, Japan*)

The coordinate regulation of iron homeostasis is largely dependent on the post-transcriptional control. However, the players and mechanisms of post-transcriptonal regulation in the iron homeostasis have not been fully understood. Our group previously found a ribonuclease, named Regnase-1, which can destabilize a set of mRNAs of pro-inflammatory cytokines, including *Il-6* and *Il-12p40*. In this study, we investigated the role of Regnase-1 in the control of iron homeostasis and anemia through the analysis of the Regnase-1-deficient mice. We found that the Regnase-1-deficient mice showed severe iron deficiency and anemia, which is partly rescued by the intraperitoneal iron supplementation. High levels of leukocytes, but not iron deficiency, observed in Regnase-1-deficient mice was rescued by the lack of lymphocytes. These findings suggest that Regnase-1 is critical for dietary iron absorption, and that this function is largely independent of the control of inflammation. To identify Regnase-1 target mRNAs responsible for the iron uptake, we conducted the transcriptome analysis in duodenum, where iron uptake takes place, and found that several iron-controlling genes, including Egln3, were up-regulated under Regnase-1 deficiency. The overexpression of Regnase-1 accelerated the decay of the Egln3 mRNA via its 3′ untranslated region. Furthermore, the administration of an Egln3 inhibitor rescued the iron deficiency in the Regnase-1-deficient mice. Taken together, these results indicate that Regnase-1 prevents the development of anemia, by regulating the duodenal iron uptake.

(COI: No)

Symposium

Symposium 1

Current somatosensory investigation reveals how skin feels the present

(March 21, 8:30～10:00, Room D)

S01-1

Simultaneous observation of skin receptors and central terminations on single primary sensory neuron

Ebara, Satomi[1]; Tonomura, Sotatsu[1]; Uta, Daisuke[2]; Furue, Hidemasa[3]; Furuta, Takahiro[4]; Kuroda, Daichi[1]; Kumamoto, Kenzo[1] (*[1]Dept Anatomy, Meiji Univ Integrative Med, Kyoto, JP; [2]Dept Applied Pharm, Grad Sch Med and Pharm Sci, Toyama Univ, Toyama, JP; [3]Dept Information Physiol, Div Neural Signaling, NIPS, Okazaki, JP; [4]Dept Morphological Brain Sci, Grad Sch of Med, Kyoto Univ, Kyoto, JP*)

Skin is innervated by primary sensory neurons known as pseudo-unipolar cells. Both peripheral and central terminations and the firing characteristics were simultaneously identified and characterized by means of intracellular labeling and recording in the rat trigeminal ganglia in vivo. Well-labeled 32 TG neurons in 32 rats were terminated as single kind of mechanoreceptors including Merkel (13), club-like (10) and lanceolate endings (7). Seven of the club-like-ending neurons never branched in their peripheral branch to the end in the follicle. They indicated relatively shorter duration at base time of action potential and showed higher frequency by air spray than the other type of endings. One of labeled lanceolate neurons distributed a 2×5 mm square receptive field on the upper eyelid. Central processes of all types of labeled neurons extended typically as far as the level of the second cervical segment of the spinal cord while emitting in excess of 20 collaterals to terminate in the trigeminal nuclei. Two samples (Merkel and club-like) showed bifurcation of the trunk axons at the level of the principal nuclei. The both trunk axons alternately gave off collaterals throughout the trigeminal tract. Those simultaneous observations on single primary sensory neurons may provide new aspects of skin sensory system.

(COI: No)

S01-2

Touch activates mechanosensitive ion channels in Merkel cells in vitro

Nakatani, Masashi[1,2]; Nelson, Aislyn M[3]; Lumpkin, Ellen A[1,4] (*[1]Columbia Univ. Med Ctr., New York, USA; [2]Keio University, Yokohama, Japan; [3]Baylor College of Medicine, Houston, TX, USA; [4]Columbia Univ., New York, USA*)

Merkel cell-neurite complexes are gentle touch receptors that mediate slowly adapting type I (SAI) responses. Since Merkel cells have been proposed to be mechanosensory cells that transduce mechanical stimuli into electrical signals that activate somatosensory neurons. Consistent with this model, conditional knockout mice that lack Merkel cells show the loss of touch-evoked SAI responses. Moreover, in vitro studies on cultured Merkel cells report calcium elevation in Merkel cells in response to swelling or membrane stretch. Previous studies support the contribution of Merkel cells to touch sensation, however, the central question of whether Merkel cells are intrinsically touch sensitive is unanswered. To tackle this problem, we performed live-cell imaging and electrophysiological recordings from mouse Merkel cells. Touch-evoked responses were monitored with either ratiometric calcium imaging or tight-seal, whole-cell recordings. Merkel cells displayed reversible calcium responses to focal displacements applied to somata. Moreover, electrophysiological recordings demonstrated mechanically activated inward currents at a negative holding potential. Merkel-cell currents adapted exponentially to sustained stimuli. Quantitative PCR indicated that Merkel cells expressed both Piezo1 and Piezo2 genes. Together, these data demonstrate that Merkel cells are intrinsically mechanosensitive in the absence of other skin cells or somatosensory neurons.

(COI: No)

S01-3

Cortical feedback control of thalamic sensory-evoked recurrent responses in rat vibrissa/barrel system

Hirai, Daichi; Shibata, Ken-ichi; Kaneko, Takeshi; Furuta, Takahiro (*Grad.Sch.Med. Kyoto Univ., Kyoto, Japan*)

Sensory gating is crucial to perception and active sensing. How and where this process takes place in is still longer unknown. Here, we identified cortical feedback could modulate temporal patterns of thalamic responses during awake sensory processing, even though their EPSPs could not directly drive spike discharges in thalamus. We broke barrel field primary somatosensory cortex (S1BF) in rats and assessed the effect on the ventral posterior medial nucleus (VPM) that projections to S1BF. After S1BF lesion, we found significant sensory-evoked rebound responses more than 50ms after stimulus onset with low-threshold spikes (LTS)-like burst in VPM and thalamic reticular nucleus (TRN), whereas smaller proportion of neurons in natural brain showed rebound responses. These results suggest thalamo-reticular circuits innately generate recurrent activity and corticothalamic feedback could suppress this activity. These results shed light on the cortical neurons as active component of sensory system and show the importance of cortical feedback in the fine control of subcortical undesirable activity.

(COI: No)

S01-4

Modulation of spinal sensory synaptic transmission by TRPV1-expressing afferent fiber

Furue, Hidemasa (*Dept Information Physiol, NIPS, Okazaki, Japan*)

Recent studies have shown that the transient receptor potential vanilloid subfamily member TRPV1, is expressed on primary afferent C fibers, and this capsaicin-activated cation channel has been proposed to play an important role in somatosensory thermal or pain signaling. However, relatively little is known about the role of the synaptic inputs from the C-fiber afferents on the spinal components of the modulation of somatic sensory transmission. We examined how capsaicin-sensitive afferent fibers modulate spinal nociceptive transmission by using in vivo and slice patch-clamp recording techniques. Superficial spinal dorsal horn (SSDH) neurons tested received excitatory monosynaptic inputs from Aδ and C fibers. Application of capsaicin presynaptically increased the frequency of the miniature excitatory postsynaptic currents (EPSCs) in most of the SSDH neurons. In GABAergic (VGAT-Venus labelled) neurons in the SSDH, capsaicin also increased the frequency of miniature EPSCs, suggesting that SSDH neurons including GABAergic interneurons make an excitatory synaptic contact with TRPV1-expressing fibers. In SSDH neurons in vivo, naturalistic sensory touch stimulation applied to the skin elicited a barrage of inhibitory postsynaptic currents (IPSCs), and the touch-evoked IPSCs were inhibited by blockade of spinal capsaicin-sensitive C fiber excitatory synaptic inputs. These results suggest that tactile cutaneous stimulation may be conveyed by a subpopulation of TRPV1-expressing C fibers, and activate inhibitory GABAergic neurons in the SSDH to reduce noxious transmission.

(COI: No)

Symposium 2

Architecture and molecular mechanisms in sensory systems

(March 21, 8:30～10:00, Room G)

S02-1

Elementary response of olfactory receptor neurons (ORNs) to odorants and its associated signaling

Yau, King-Wai (*Dept Neuroscience, Sch of Med, Johns Hopkins Univ, Baltimore, Maryland, United States of America*)

This talk will summarize some of our past key experiments on olfactory transduction. The sense of smell begins with odorant molecules binding to odorant receptors on the membrane of ORN cilia, thereby activating a G protein, Golf, and the downstream effector enzyme, an adenylyl cyclase (ACIII). As a result, the intracellular cAMP concentration rises and opens a cyclic-nucleotide-gated, non-selective cation channel to depolarize the cell. With repeated, identical weak odorant pulses and variance analysis to study the ensemble of elicited responses, we found in both frog and mouse ORNs that the unitary response was surprisingly constant, despite large variations in the macroscopic sensitivity, across ORNs. We infer that an odorant-binding event has a very low probability of activating sensory transduction at all. Thus, even when successful, the resulting unitary response apparently involves a single active G alpha olf-ACIII molecular complex. This low signal amplification is in contrast to rod phototransduction in vision, where each photoisomerized rhodopsin molecule is known to produce substantial amplification by activating many downstream G protein molecules, hence many effector-enzyme molecules. From the action-potential firing, we estimated that perhaps 20 or so odorant-binding events that successfully triggered transduction in an frog or mouse ORN will lead to signaling to the brain. If time permits, some unpublished recent experiments will also be discussed.

(COI: No)

S02-2

Respiration rhythm and olfaction

Mori, Kensaku; Manabe, Hiroyuki; Narikiyo, Kimiya (*Dept Physiol, Grad Sch Med, Univ of Tokyo, Japan*)

In land mammals including humans, olfactory perception critically depends on discrete respirations, each consisting of an inhalation phase followed by an exhalation phase. During the inhalation phase, odorants are drawn into the nasal cavity and activate olfactory sensory neurons in the nasal sensory epithelium. Thus the central olfactory system is driven from the external odor stimuli and processes the olfactory sensory information during the inhalation phase. On the contrary, the central olfactory system is temporarily isolated from the external odor world during the exhalation phase. Therefore respiration rhythm plays a key role orchestrating the information processing mode across a number of regions in the central olfactory system, which includes the olfactory bulb and numerous areas of the olfactory cortex. We made electrophysiological recordings of local field potentials and single-unit activities in behaving rats while the respiration pattern of rats was monitored by a thermocouple placed in the nasal cavity. We report dynamic changes in the operation mode of information processing in the central olfactory system during the inhalation-exhalation sequence of respiration.

(COI: No)

S02-3

Does calmodulin modulate the functions of the TMEM16 calcium-activated chloride channels?

Chen, Tsung-Yu (*Center for Neuroscience and Department of Neurology, University of California, Davis, USA*)

TMEM16 gene family members, TMEM16A and TMEM16B, have recently been identified to be the calcium-activated chloride channels (CaCCs) in various sensory and respiratory tissues in the nose and are important for the olfactory functions of vertebrate animals. It has been known that CaCCs play a critical role in amplifying the odorant-induced inward current through the calcium-permeable cyclic nucleotide-gated channels (CNC) in olfactory receptor neurons. In the odorant signal transduction process, modulation of the olfactory CNC by calcium-calmodulin has been known to be critical for olfactory sensory adaptation. Whether calmodulin modulates the TMEM16 family members is, however, controversial. Biochemical experiments from different laboratories showed controversial results regarding calmodulin binding to TMEM16 family members. Functionally, calmodulin was thought by some investigators to be required for the activation of the TMEM16 CaCCs by calcium, although experiments from other laboratories including ours suggested that activation of TMEM16A and TMEM16B channels does not require calmodulin. Finally, calcium-calmodulin was also shown to alter the anion permeability of TMEM16A channel. However, by directly applying calcium-calmodulin to the intracellular side of excised inside-out membrane patches, we are unable to observe this calcium-calmodulin effect on the anion permeation of TMEM16A channel, although the calmodulin used in our laboratory rigorously inhibits the olfactory CNC formed by subunit CNCA2.

(COI: No)

S02-4

Measurement of metabolic activity of single mammalian photoreceptors

Koutalos, Yiannis; Adler, Leopold; Chen, Chunhe (*Department of Ophthalmology, Medical University of South Carolina, Charleston, USA*)

In vertebrate rod photoreceptors, all-trans retinal is released by photoactivated rhodopsin following light excitation. All-trans retinal is then reduced to all-trans retinol, in a reaction that requires NADPH. The extent of conversion of all-trans retinal to all-trans retinol is a measure of the NADPH-generating capacity of the cell. We have used the fluorescence of all-trans retinal and all-trans retinol to monitor their levels in single rod photoreceptor cells with fluorescence imaging. Rod photoreceptors were isolated from the retinas of dark-adapted mice and human donor eyes. All-trans retinal was generated either endogenously by photoactivating rhodopsin with long-wavelength light (longer than 530 nm), or supplied exogenously with bovine serum albumin as carrier. The fluorescence signals of all-trans retinal and all-trans retinol were distinguished on the basis of the large difference in their absorption spectra. Conversion of all-trans retinal to all-trans retinol was measured from the ratio of the fluorescence intensities excited by 340 and 380 nm light (emission longer than 420 nm). Experiments were carried out at 37 ^{0}C. We find that NADPH generation requires the presence of extracellular metabolic substrates, with glutamine supporting NADPH generation to levels comparable to those of glucose. The results suggest that in rod photoreceptors mitochondria-linked pathways can generate substantial amounts of NADPH. This allows the cells to utilize a variety of metabolic substrates to maintain viability under transient nutrient shortages.

(COI: No)

S02-5

Spatial structure of actin cytoskeletons in retinal pigment epithelial and photoreceptor cells

Usukura, Jiro (*Grad.Sch.Sci.Nagoya Univ., Nagoya, Japan*)

The spatial organization of cytoskeletal actin filaments in retinal pigments epithelial and photoreceptor cells were studied by high voltage TEM (1000 KV), high resolution SEM and freeze etching method. Actin cytoskeletons have been investigated so far exclusively with fluorescent light microscopy by Phalloidin staining or GFP tag method using culture cells. Therefore, actin filaments in cytoplasm, in particular, peri-nuclear region in real tissue cells were not observed enough yet. In order to detect real spatial structure of actin cytoskeleton, unroofed whole pigment epithelial cells cultured from monkey eye were applied to 1000 KV TEM. Our innovative methods detected incredibly more abundant actin filaments than in fluorescent microscopy. Interestingly, no stress fibers were found in spite of culture cells. Remarkable amount of actin filaments occupying the entire cytoplasm extended in all directions with aggregation and dispersion to form meshwork, and eventually divided cytoplasmic space into several domains. These actin filaments contained specific anti-myosin II antibody binding site. However, myosin filaments were not recognized on actin filaments under freeze-etching electron microscopy. Therefore, myosin II might attach to actin filaments as a single molecule or non-detectable short filaments consisting of a few molecules. In photoreceptor cells, actin filaments were observed widely in inner segments from ellipsoid region to synaptic area, though actin filaments in the outer segment were found only in tip of connecting cilium.

(COI: No)

Symposium 3

Frontiers in mitochondrial dynamics and pathophysiology

(March 21, 8:30～10:00, Room H)

S03-1

Physiological roles of mitochondrial fusion and fission in mice development

Ishihara, Naotada (*Inst. Life Sci., Kurume Univ., Kurume, Japan*)

Mitochondria are highly dynamic organelles that change their morphology during cellular signaling, differentiation and pathogenic condition. Several types of GTPase proteins regulate dynamic morphogenesis of mitochondria, although their physiology have been poorly understood. To assess the physiological role of mitochondrial fission, we generated knock-out (KO) mice of mitochondrial fission factor dynamin-related protein (Drp)1 by using Cre-loxP system. Mice lacking the mitochondrial fission GTPase Drp1 have developmental abnormalities, and die after embryonic day 12.5. Neural cell-specific Drp1-deficient mice die shortly after birth due to brain hypoplasia with apoptosis, due to failed proper distribution of mitochondria. In various developmental stages and pathogenic conditions, mitochondrial morphology is highly changed, and the regulated mitochondrial fission might have important roles in tissue differentiation. We also found a novel role of mitochondrial fission in distribution of mtDNA. Mammalian cells typically contain thousands of copies of mtDNA assembled into hundreds of nucleoid structures. We analyzed the dynamic features of the nucleoids in terms of mitochondrial membrane dynamics, and found that nucleoids in Drp1-deficint cells were enlarged by their clustering within hyperfused mitochondria. The dynamics of nucleoid structures regulated by mitochondrial fission contributed to cristae reformation, proapoptotic status of mitochondria, and thus the tissue differentiation in vivo.

(COI: No)

S03-2

Role of mitochondrial ubiquitin ligase MITOL in mitochondrial dynamics and diseases

Yanagi, Shigeru (*Tokyo Univ. Pharm. Life Sci., Tokyo, Japan*)

We have previously identified mitochondrial ubiquitin ligase, MITOL (also known as March5), which regulates mitochondrial dynamics through the ubiquitination of mitochondrial fission factor Drp1. Subsequently, we reported that MITOL ubiquitinated and attenuated cell toxicity of unfolded proteins accumulated in mitochondria such as mutant SOD1 and expanded polyglutamine proteins which cause neurodegenerative disorders, suggesting the involvement of MITOL in mitochondrial quality control and pathogenesis of neurodegenerative diseases. To further understand the role of MITOL in mitochondria, we searched for physiological substrates for MITOL and succeeded to identify microtubule-associated protein 1B-light chain 1 (MAP1B-LC1) and mitofusin2 (Mfn2). Recently, we report that MITOL plays a protective role against nitrosative stress-induced mitochondrial dysfunction mediated by MAP1B-LC1 in neuronal cells, and that MITOL is required for ER-mitochondria interaction via Mfn2 activation. In the symposium, I will show several unpublished data obtained from analyses of MITOL-deficient MEFs and mice, and discuss the role of MITOL in mitochondrial dynamics and diseases.

(COI: No)

S03-3

Mitochondrial dynamics in damaged neurons

Kiryu-Seo, Sumiko; Kiyama, Hiroshi (*Grad.Sch.Med.Nagoya Univ., Nagoya, Japan*)

The physiological relevance of mitochondrial fission in damaged neurons remained to be determined, although numerous studies observe fragmented mitochondria in damaged neurons of neurodegenerative disease and traumatic injury models. To address this issue, attempts have been made to elucidate the functional consequences of mitochondrial fission under physiological and pathological conditions in vivo. In this symposium, we will introduce the recently established unique bacterial artificial chromosome transgenic (BAC Tg) mice, in which mitochondria are labeled with GFP and cre recombinase is expressed simultaneously in injury specific manner, and will discuss the critical role of mitochondrial fission in damaged neurons in vivo. The BAC Tg mice demonstrate that GFP-labeled shorter mitochondria are actively transported to replace pre-existing GFP-negative longer mitochondria in regenerative injured motor axons, suggesting the enhanced activity of mitochondrial fission after nerve injury. Crossing the BAC Tg mice with the dynamin-related protein 1 (Drp1) floxed mice succeeds in the ablation of mitochondrial fission specifically in injured motor neurons. The injury-inducible Drp1 knockout mice show the microglial activation in the proximity of injured neurons from earlier stage and the elongated or gigantic mitochondria with lower quality, prior to neuronal death and axonal degeneration. Thus, mitochondrial fission could be an acute defensive response for injured neurons to satisfy huge amounts of energy demands and to maintain mitochondrial and neuronal integrity.

(COI: No)

S03-4

How dysfunction of mitochondrial quality control causes Parkinson's disease

Matsuda, Noriyuki (*Protein Metabo Pro, Tokyo Metro Inst of Med Sci, Japan*)

PINK1 and PARKIN have been identified as the causal genes responsible for hereditary Parkinson's disease (Kitada et al., 1998). To date, there is significant evidence supporting a functional link between PINK1, Parkin and mitochondrial quality control. PINK1 is a serine/threonine kinase that specifically accumulates on and is activated by mitochondria with a decreased membrane potential. PINK1 then activates the latent ubiquitin ligase (E3) activity of Parkin and recruits it to depolarized mitochondria (Matsuda et al., 2010; Narendra et al., 2010). Parkin catalyzes ubiquitin transfer to various substrates on depolarized mitochondria. As a consequence, inferior mitochondria with low membrane potential are quarantined and degraded via the proteasome and autophagy.

We and other groups have revealed the mechanistic insights into PINK1-mediated Parkin activation recently. Parkin is an intramolecular auto-inhibitory E3 that usually has its catalytic Cys431 core occluded by a RING0 domain. PINK1 phosphorylation of Ser65 in the ubiquitin-like domain of both Parkin and ubiquitin triggers removal of Parkin autoinhibition by phosphorylated ubiquitin, which then results in the conversion of phosphorylated Parkin to the fully active form (Kane et al., 2014; Koyano et al., 2014). On the other hand, while various models for the recruitment process have been proposed, all inadequately explain the accumulated data, thus the molecular basis for PINK1 recruitment of Parkin remains to be fully elucidated. We are now revealing the molecular mechanism of Parkin recruitment, and the newest results will be presented.

(COI: No)

Symposium 4

Dynamic aspects of microscopic localization, stoichiometry and function of membrane protein complexes

(March 21, 8:30～10:00, Room I)

S04-1

Quantitative localization of bio-molecules in the neuronal plasma membrane by immuno-electron microscopy

Fukazawa, Yugo[1]; Shigemoto, Ryuuichi[2] (*[1]Div Cell Biol and Neurosci, Sch Med, Univ Fukui, Fukui, Japan; [2]IST Austria*)

The dendrites of neurons, major receiving part of synaptic input, plays roles in more than integrating and passing received postsynaptic potential down to the soma and axon initial segment at where the action potential is generated. They have ability to shape and integrate individual potentials thereby involved in regulation of neurons behavior. These abilities are achieved by orchestrated actions of transmitter receptor, voltage-gated ion channels and ion pumps expressed in the dendritic plasma membrane (PM). Thus, to understand mechanisms underlying neuronal excitability, it is crucial to identify molecular species expressed in a target neuron and quantity (density) and their distribution of each molecule in the PM. Toward this goal, we have been investigating distribution of several key molecules such as ionotropic receptors and ion channels in pyramidal cells in the hippocampus by means of quantitative immuno-electron microscopic approaches. We have just started to analyze distribution of neuron specific Na/K ATPase which is responsible for maintenance of resting membrane potential. In this presentation, we will introduce our recent results including these from other ongoing analysis.

(COI: No)

S04-2

Spatial Regulation of $GABA_AR$ Synaptic Structure by Glutamate and Calcium

Bannai, Hiroko[1,2]; Niwa, Fumihiro[2]; Triller, Antoine[3]; Mikoshiba, Katsuhiko[2] (*[1]Div Biol Sci, Grad Sch Sci, Nagoya Univ, Nagoya, Japan; [2]RIKEN BSI, Wako, Japan; [3]IBENS, Paris, France*)

GABAergic synaptic transmission regulates brain function by establishing the appropriate excitation-inhibition (E/I) balance in neural circuits. The structure and function of GABAergic synapses are sensitive to destabilization by impinging neurotransmitters. However, signaling mechanisms that promote the restorative homeostatic stabilization of GABAergic synapses remain unknown. Here, we characterized a signaling pathway that promotes the stability of $GABA_A$ receptor ($GABA_AR$) postsynaptic organization by quantum dot-single particle tracking. Slow metabotropic glutamate receptor signaling activated IP_3 receptor-dependent calcium release and protein kinase C phosphorylation to promote $GABA_AR$ clustering and GABAergic transmission. This $GABA_AR$ stabilization pathway counteracted the rapid cluster dispersion caused by glutamate-driven NMDA receptor-dependent calcium influx and calcineurin dephosphorylation, including in conditions of pathological glutamate toxicity. These findings show that glutamate activates distinct receptors and spatiotemporal patterns of calcium signaling for opposing control of GABAergic synapses. This mechanism of inhibitory synaptic stabilization will enable therapies to restore E/I imbalance in major brain diseases.

(COI: Properly Declared)

S04-3

Impact of transient homodimers: the basic units for signaling and domain formation found for both GPCRs and GPI-anchored receptors

Kusumi, Akihiro (*WPI-iCeMS and Inst. for Frontier Med. Sci. Kyoto Univ.*)

Single-molecule tracking techniques applicable to live cells are now providing researchers with the unprecedented ability to directly observe molecular behaviors in the plasma membrane (PM) of live cells. Using ultra-speed simultaneous two-color single-molecule colocalization and single-molecule FRET, we found that class-A G-protein-coupled receptors (GPCRs) and GPI-anchored receptors (GPI-AR) form transient homodimers with lifetimes on the order of 0.1 s and that these transient dimers are critical for triggering some of the signaling pathways.
(1) We fully determined the dynamic monomer-dimer equilibrium of prototypical GPCRs, N-formyl peptide receptor and adrenergic receptor, I,e, the equilibrium constant and dimer formation-dissociation rate constants, indicating that, under physiological expression conditions at 37 degrees, 42 and 95% of the molecules, respectively, exist as dimers in the live-cell PM at any moment These transient dimers triggered the steady-state signals characteristic of GPCRs.
(2) Meanwhile, GPI-ARs continually form transient homo-dimers through ectodomain protein interactions, stabilized by raft-lipid interactions (termed homo-dimer rafts). When CD59 was ligated, a few homo-dimer rafts turned into a stable oligomer rafts, which triggered intracellular Ca2+ responses. Transient homo-dimer rafts are most likely one of the basic units for organization and function of raft domains containing GPI-ARs.
In conclusion, these results suggest that taking advantage of transient homo-dimers might be a basic strategy for the receptor-based signal transduction in the PM.

(COI: No)

S04-4

Expression density dependent changes of the stoichiometry and function of ion channel complexes

Kubo, Yoshihiro[1,2]; Kitazawa, Masahiro[1,2]; Nakajo, Koichi[1,2] (*[1]Div Biophys & Neurobiol, Natl Inst Physiol Sci, Okazaki, Japan; [2]Physiol Sci, SOKENDAI, Hayama, Japan*)

It has been known that many ion channels do not stand alone but function forming molecular complex with other accessary subunits. Biochemical analyses provide us with information as to the molecular identity in the complex as well as the bulk average of the stoichiometry. However, the detail of the stoichiometry had not been analyzed. Ulbrich et al (Nat Methods, 2007) applied a single molecule imaging technique to determine the stoichiometry of ion channel complexes. It is possible to evaluate the number of subunits in the complex by counting the number of bleaching steps of the fluorescent protein tagged to the subunits by single molecule imaging. In this presentation, two examples from our achievements are introduced. (1) KCNQ1/ KCNE1 K^+ channel complex plays important roles in the cardiac rhythmic beating, and it had been generally accepted that the stoichiometry is 4:2. We applied single molecule subunit counting technique and demonstrated the presence of 4:4 complex. Furthermore, we observed that the stoichiometry varies depending on the relative expression density (Nakajo et al, PNAS 2010). (2) Kv4 K^+ channel plays roles in the neuronal and cardiac functions. It is well accepted to form a molecular complex with accessary subunits such as KChIP and DPP. We analyzed Kv4.2/KChIP4 complex and observed that the stoichiometry changes with the increase in the relative expression level of KChIP4, as if KChIP4 binds to the 4 independent sites with no preferred stoichiometry (Kitazawa et al, JBC 2014).

(COI: No)

Symposium 5

The strategies aimed at maintenance of tissue perfusion ～Regulation of cardiomyocyte apoptosis and angiogenesis～

(March 21, 8:30～10:00, Room J)

S05-1

TCTP expression level may be critical for protection against apoptosis of cardiomyocytes and development of cardiac dysfunction

Fujita, Takayuki; Cai, Wenqian; Hidaka, Yuko; Jin, Hui-lin; Hasegawa, Nozomi; Suita, Kenji; Ishikawa, Yoshihiro (*Cardiovascular Research Institute, Yokohama City Univ. Yokohama, Japan*)

Translationally Controlled Tumor Protein (TCTP), one of the anti-apoptotic proteins, is ubiquitously expressed in human tissues. TCTP is reported to exert anti-apoptotic effect through direct interaction with p53 and MCL-1. TCTP works as a p53 inhibitor. In addition, TCTP inhibits degradation of MCL-1, an anti-apoptotic protein, thereby maintaining its expression level.Doxorubicin (DOX) is a widely used chemotherapeutic agent for cancer therapy. However, its clinical usage has been limited by its serious cardiotoxicity. We found that after treatment of DOX, protein expression of TCTP was significantly decreased both in cultured rat ventricular cardiomyocytes and mouse heart. Moreover, down-regulation of TCTP by siRNA induced apoptosis of cardiomyocytes. In addition, our recent studies showed that Dihydroartemisinin (DHA), a TCTP down-regulating agent, induces apoptosis of cardiomyocytes and cardiac dysfunction. Consistently, cardiac specific overexpression of TCTP prevents both of doxorubicin-induced and DHA-induced apoptosis of cardiomyocytes and cardiac dysfunction. These findings indicate that TCTP expression level may be critical for protection against apoptosis of cardiomyocytes and development of cardiac dysfunction. TCTP may be a novel potent therapeutic target for heart failure.

(COI: No)

S05-2

Disruption of Epac1 decreases phosphorylation of phospholamban and protects the heart against chronic catecholamine stress

Okumura, Satoshi (*Department of Physiology, Tsurumi University, Japan*)

Protein kinase A (PKA) phosphorylates multiple molecules involved in calcium (Ca^{2+}) handling in cardiac myocytes, and is considered to regulate β-adrenergic receptor-mediated enhancement of cardiac contractility. However, this paradigm has been challenged by the recent identification of Epac (exchange protein activated by cAMP), which is activated by cAMP independently of PKA. Epac1-null mice (Epac1KO) showed decreased cardiac contractility with decreased phospholamban (PLN) phosphorylation at serine-16, the major PKA-mediated phosphorylation site. Intracellular storage of Ca^{2+} was decreased in Epac1KO. However, PKA expression remained unchanged and isoproterenol improved cardiac contractility. In contrast, direct activation of Epac led to increased phospholamban phosphorylation at serine-16 in cardiomyocytes and this phosphorylation is considered to involve Epac1/PLC/PKC. More importantly, chronic isoproterenol infusion (60mg/kg/day for 7days) induced a similar degree of cardiac hypertrophy in Epac1KO and WT, but subsequent cardiac dysfunction was prevented in Epac1KO, in association with decreased cardiac myocyte apoptosis and fibrosis. Epac1 is an important regulator of phospholamban phosphorylation, independently of PKA, and appears to regulate cardiac responsiveness to chronic catecholamine stress.

(COI: No)

S05-3

Visualization of Angiogenesis

Morikawa, Shunichi (*Tokyo Women's Med. Univ., Tokyo, Japan*)

Angiogenesis is an important event not only in normal developmental process, but also in abnormal pathological processes such as malignant tumors. In tumors, angiogenesis is a serious problem since it helps proliferation of tumor cells by supplying them oxygen and nutrients and also helps metastatic spread of tumor cells via blood stream. Therefore, in tumor therapy, suppression of the angiogenesis need to be taken in account. On the contrary, in the therapy of ischemic diseases such as myocardial infarction, re-perfusion of blood in ischemic area is necessary; promotion of angiogenesis is conversely important in this case. To cope with the diseases, we need to take a closer look into the actual scene of angiogenesis and study the cellular mechanism of it. For this purpose, clear and detailed visualization of angiogenesis is required in the first place. In the session, representative images of the actual scene of angiogenesis will be presented; they include fine-structural images of newly forming vessels revealed by ultrathin sections, or three-dimensional (3D) images that have been re-constructed from serial cross sections. By the 3D images, we can see the whole appearance of newly forming vessels that we cannot grasp by thin sections. From these images, we can assess how endothelial cells proliferate and grow, and how pericytes behave during angiogenesis. We can also see an increased permeability and abnormal coverage of basement membrane of newly forming vessels.

(COI: No)

S05-4

Novel mechanisms involved in the endothelial differentiation of arteries and veins

Saito, Erina[1,2]; Isogai, Sumio[1]; Kimura, Eiji[1]; Shimoda, Hiroshi[2]; Hitomi, Jiro[1] ([1]*Iwate Med Univ., Iwate, Japan;* [2]*Grad.Sch.Med.Hirosaki Univ., Aomori, Japan*)

It had been long believed that flow dynamics play crucial role in the capillary bed to determine the arterial venous identity, but recent studies revealed that genetic cues induce the cell fate before blood flow initiates. Since the findings, vascular biologists focused their attention intensely upon the expression of artery and vein specific genes in the vascular development. It was inevitable to detect when and how the molecular differences reflect the phenotypic differences. These molecular evidences forced us to reconsider the biological mechanisms involved in the differentiation of endothelial cells from the mesoderm, acquisition of arterial or venous identity and tube formation. To verify the differentiation of endothelium from the mesoderm, we captured the time lapse movies following whole the morphogenetic process of the primary vascular system for the brain in vivo using fli1 EGFP transgenic zebrafish. The arterial angioblasts coalesced into the small luminized cluster, and the tightly adhered angioblasts generated seamless major artery by so called cord hollowing mechanism. On the contrary, the venous angioblasts never coalesced, but taking a leaf-like shape individually, encircle a wide lumen. This patchwork like formation manner and a larger number of cell participated with the formation of vein allowed to form a wide seamed vessel. Our results suggest that endothelial precursors of major cerebral arteries and veins have completed each individual morphogenesis using different mechanism through vasculogenesis and angiogenesis.

(COI: No)

S05-5

Vasohibin-2 modulates tumor onset by normalizing tumor angiogenesis

Kitahara, Shuji (*Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, USA*)

Vasohibin-2 (VASH2) has been identified as an extrinsic and vascular endothelial growth factor independent angiogenic factor that is highly expressed in tumor cells. In the present study, we aimed to find whether pre-existing vascular changes can be used to predict tumor transformation as benign or malignant. We sought to characterize the microvascular changes and tumor development in the intestinal tract of $Apc^{Min/+}$ mice and the $Apc^{Min/+}/Vash2^{-/-}$ mice. $Apc^{Min/+}$ mice provide a unique orthotopic model for the development of spontaneous adenomatous polyposis and subsequent carcinomas-termed the adenoma-carcinoma sequence. $Apc^{Min/+}$ mice were mated with $Vash2^{-/-}$ mice with a mixed C57BL/6 background and the resulting pups were screened for the Min mutation and for the $Vash2^{-/-}$ gene by PCR. The intestinal tumors of $Apc^{Min/+}$ mice and the $Apc^{Min/+}/Vash2^{-/-}$ mice were removed and either frozen or epon-embedded for subsequent analyses. $Apc^{Min/+}/Vash2^{-/-}$ mice showed a significant decrease in the number of polyps in the small intestine. Furthermore, functional tumor blood vessels were decreased, and pericyte coverage tumor blood vessels were increased. We may propose that VASH2 modulates the onset of tumors in the gastrointestinal tract by regulating tumor angiogenesis.

(COI: No)

S05-6

The function of platelet factor CLEC-2 in the hematopoietic stem cell niche

Ishizu, Ayako; Suda, Toshio (*National University of Singapore, Singapore*)

The bone marrow (BM) niche governs the integrity of hematopoietic stem cells (HSCs). While vascular and perivascular cells constitute potent niches, we recently identified mature megakaryocytes (Mk) as an independent niche component. Ontogenetically, the vascular and hematopoietic system development intertwine; molecular defects in platelets result in blood and lymphatic vessel dis-segregation and hematopoietic abnormalities. We explored the function of one of these markers, platelet activation receptor C-type lectin like receptor-2 (CLEC-2) in hematopoiesis. Specific deletion of CLEC-2 on Mk lineages (*PF4Cre:CLEC-2$^{floxed/floxed}$; Clec2$^{Mk\delta/\delta}$*) confined the function of BM HSCs; *Clec2$^{Mk\delta/\delta}$* HSCs showed reduced cell cycle quiescence and lower post-transplantation chimerisms in competitive BM transplantation assay. *Clec2$^{Mk\delta/\delta}$* mice exhibited abnormal HSC mobilization to the peripheral blood and splenomegaly due to extramedullary hematopoiesis reflecting a failure of *Clec2$^{Mk\delta/\delta}$* BMs to sustain HSCs. Although *Clec2$^{Mk\delta/\delta}$* Mk progenitors matured without arrest in normal ploidy, sorted *Clec2$^{Mk\delta/\delta}$* Mks failed to maintain HSC populations in vitro. CLEC-2 deficient Mks exhibited decreased production of various niche factors including Thrombopoietin (Thpo). Furthermore, despite presenting thrombocytopenia, serum and BM Thpo levels were remarkably low in *Clec2$^{Mk\delta/\delta}$* mice. We identify CLEC-2 as an Mk specific molecule that formulates a niche for the maintenance of HSCs through the modulation of Thpo production. Our study provides insight into the close relation between the vascular system and Mks regarding hematopoiesis.

(COI: No)

Symposium 6

Front in progress on aerospace medicine and biology

(March 21, 14:00～15:30, Room B)

S06-1

Response of osteoclasts in the regenerating scales of goldfish under microgravity during space flight

Ikegame, Mika[1]; Tabata, Makoto[2]; Hattori, Atsuhiko[3]; Suzuki, Nobuo[4] (*[1]Dept.Oral Morph., Okayama Univ.Grad.Sch.Med.Dent.Pharm., Okayama, Japan; [2]Grad.Sch. Tokyo Med.Dent.Univ., Tokyo, Japan; [3]Coll.of Lib.Arts Sci., Tokyo Med.Dent.Univ., Chiba, Japan; [4]Inst.Nat.Environ.Technol., Kanazawa Univ., Ishikawa, Japan*)

Microgravity during space flight leads to rapid bone loss. However, the cellular mechanisms underlying this phenomenon remain unclear. Teleost scale is a calcified tissue which is similar to mammalian bone tissue in many aspects. We investigated the response of osteoclasts during space flight using goldfish scales. Regenerating scales were incubated for 86 h under microgravity (F-μg) or artificial 1 g (F-1g) at the International Space Station. We also performed three-dimensional clinostat experiments to examine immunohistochemical localization of receptor activator of nuclear factor kappa-B ligand (RANKL), which is a crucial factor for the development and activation of osteoclasts, in the scales under modeled microgravity. A significant increase in osteoclast's nucleus number and the size of actin-ring was observed in F-μg group compared to F-1g group. Real-time PCR analysis demonstrated the up-regulation of RANKL gene expression in F-μg group. Furthermore, the immunohistochemical localization of RANKL was increased under the modeled microgravity. These results suggest that one of the mechanisms of bone loss during space flight is the stimulated activity of osteoclasts via up-regulation of RANKL expression.

(COI: No)

S06-2

Bone formation and resorption under microgravity in medaka rearing in International Space Station (ISS)

Takano, Yoshiro (*Grad.Sch.Med.Dent., TMDU.Tokyo, Japan*)

Molecular mechanisms underlining loss of bone mineral density (BMD) during space flight still remain unclear. With strong supports from JAXA and JSF, we were fortunate to have an opportunity to examine structure and function of bone-related cells in medaka rearing under microgravity in the ISS up to 2 months. We focused on the pharyngeal bone, which is the site of dynamic bone remodeling.

Method: To investigate the activity of osteoclasts (OC) and osteoblasts (OB), we established the TRAP promoter-GFP/Osterix promoter-DsRed double Tg medaka and reared in the aquatic habitat at "Kibo", Japanese experimental module in the ISS. Fish were either fixed with 4 % PFA at day 16 and day 58, or preserved in RNAlater at day 2 and day 62. BMD was measured by Soft X-ray and μCT and the OC and OB activities by confocal microscopy and histological methods. The gene expression level was examined by the whole transcriptome analysis using HiSeq.

Results and Discussion: In 16 days-rearing group, the total volume of GFP and DsRed expressing cells decreased respectively by 49% and 68% ($p<0.05$) while the OC/OB volume ratio elevated 1.45 folds compared to 1G control ($p<0.05$). In 58 days group, BMD decreased by 30% compared to time-matched 1G control without notable changes in enzymatic activities and ultrastructure in OC and OB cells. These data indicate that, under microgravity, while OC and OB become less active in 16 days, the OC/OB ratio increases and leads to decreased BMD in later periods.

Conclusion: Taken together, we conclude that microgravity exerts a condition similar to the low bone-metabolic rotation osteoporosis.

(COI: No)

S06-3

Cardiovascular responses to acceleration in rats

Maruyama, Satoshi[1]; Nishida, Yasuhiro[2] (*[1]Aeromedical Laboratory, Japan Air Self-Defense Force; [2]Department of Physiology, National Defense Medical College*)

During flight, various aircraft maneuvers often produce sustained acceleration (+Gz stress) during pitch and banked turns. Acceleration of +4Gz or greater values can cause deficiency in retinal and cerebral perfusion and results in visual field abnormality such as gray out, black-out or loss of consciousness. Peripheral and central vision disturbances are caused by a reduction in peripheral retinal perfusion. These visual disturbances precede reduced brain perfusion and are followed by a loss of consciousness. Understanding of cardiovascular and autonomic responses to +Gz stress is very important to secure flight safety. We have investigated the effects of +Gz on the cardiovascular system using rats and found some important results: (1) +5 Gz stress may suppress baroreflex response and subsequently cause increased hypotension; (2) brain blood flow response to Gz exposure depends on the brain loci that are affected; and (3) repetitive lower acceleration (+1.5Gz) exposures eliminate in decrease of cerebral arterial pressure and reduction of cortical oxygen concentration. We also describe some basic physiological phenomenon affected by sustained acceleration.

(COI: No)

S06-4

Hypoperfusion-reperfusion injury and reactive oxygen species: spin resonance analyses

Tokumaru, Osamu[1]; Ogata, Kazue[1]; Kitano, Takaaki[2]; Yokoi, Isao[1] (*[1]Dept Neurophysiol, Oita Univ Fac Med, Oita, Japan; [2]Dept Anesthesiol, Oita Univ Fac Med, Oita, Japan*)

Under high Gz acceleration environment (head-to-foot inertial force), fighter pilots are subject to hypoperfusion in brain. Decrease in tissue oxygen concentration leads to breakdown of ATP to hypoxanthine and activation of xanthine oxidase. On reperfusion, resupply of oxygen leads to production of superoxide anion radicals, from which many kinds of free radical species are produced in a series of chain reactions, resulting in tissue damage.By phosphorous nuclear magnetic resonance (NMR) spectroscopy, it is possible to measure high-energy phosphates, phosphocreatine (PCr) and ATP in rat brain slices. The observation revealed decrease in PCr and ATP during ischemia and delayed recovery of energy metabolic status compared with those of oxygen and glucose. Free radicals can be directly observed by electron spin resonance (ESR) spectroscopy. Using ESR, we have evaluated direct scavenging activity of antioxidants against multiple kinds of free radicals. Since the life time of free radicals are short (μs-ms), it is difficult to observe free radical production in living tissue. We have tried to detect it only in vain. In clinical settings, decrease in vitamin C radicals in serum is observed in post-operative patients. Application of those "spin resonance analyses" will make it possible to better understand the pathophysiology including change in energy metabolism and production of reactive oxygen species in rat brain exposed to high Gz environment, contributing to the improvement of the countermeasures and flight safety.

(COI: No)

Symposium 7

Recent advances in the research on the trigeminal ganglion

(March 21, 14:00～15:30, Room D)

S07-1

Pharmacological Action of Eugenol: Go beyond Dental Clinic

Oh, Seog Bae (*Dept Neurobiol and Physiol, Sch Dent, Seoul National Univ, Seoul, South Korea*)

Eugenol, an active ingredient of essential oil extracted from cloves and other herbs, is used extensively in dentistry for the analgesic purpose. However, the molecular mechanism underlying the analgesic activity of eugenol is mostly unknown. A series of investigation in our lab have revealed that eugenol modulates various ion channels that are responsible for nociception, generation of neuronal spikes, and synaptic transmission in the trigeminal system. Pharmacological action of eugenol includes inhibition of action potential firing by reducing voltage-gated sodium, calcium and potassium channels in the trigeminal ganglion neurons. In addition, we found eugenol inhibits hyperpolarization-activated cyclic nucleotide-gated (HCN) channels that play a crucial role in mechanical allodynia in neuropathic pain state. Concurrently, eugenol successfully reversed mechanical allodynia in experimental trigeminal neuropathic pain animal, at much less dose than it blocks sodium channels. Modulation of $P2X_3$ receptor, an ionotropic ATP receptor, might be another mechanism by which eugenol exerts its analgesic activity. Activation of TRPV1 and TRPA1 by eugenol might also contribute to the analgesic effect, since pharmacological activation of TRPV1 and TRPA1 has been shown to produce biphasic action of initial pungent and sustained inhibition of nociception. In conclusion, eugenol is a natural compound that displays a number of pharmacological properties with therapeutic potential for versatile analgesic applications.

(COI: No)

S07-2

Modulatory mechanisms of inflammatory nociceptive signals in the trigeminal ganglia

Takeda, Mamoru (*Lab Food Physiol Sci, Sch life Enviro Sci, Azabu Univ, Kanagawa, Japan*)

Peripheral tissue inflammation can alter the properties of somatic sensory pathways, causing behavioral hypersensitivity and resulting in increased responses to pain caused by noxious stimulation (hyperalgesia) and normally innocuous stimulation (allodynia). Although no synaptic transmission has been found in the primary sensory ganglia, it has been discovered that the activity of neighboring neurons elicits a functional cross-excitation in the somata of affected sensory neurons under normal conditions, indicating that non-synaptically released diffusible chemical messengers modify the neuronal excitability of the sensory ganglia (Amir and Devor 2000). Non-synaptically released chemical mediators were derived from both the neurons and the satellite glia (e.g. neuron-neuron and neuron-glia interactions). More recent studies have suggested that modulation of neuronal excitability within sensory ganglia, including trigeminal ganglia may trigger chronic pain via the autocrine/paracrine mechanism, and this augmented excitability of the primary afferent neurons may also cause the development of changes in the central pain-signaling neurons (central sensitization).Therefore, the present talk focuses on the modulation of the neuronal signal by cross-talk in the trigeminal ganglia, particularly with regard to its contribution to inflammatory pain, and discusses the potential therapeutic target for the prevention of hyperalgesia/allodynia.

(COI: No)

S07-3

Involvement of intra-trigeminal ganglionic communication in ectopic orofacial pain

Shinoda, Masamichi; Iwata, Koichi (*Dept Physiol, Sch Dent, Nihon Univ, Tokyo, Japan*)

Pathological orofacial pain which spreads to a wide area in the trigeminal territory occurs with orofacial inflammation or trigeminal nerve injury. However, the peripheral mechanisms underlying such ectopic orofacial pain remain unclear. We investigated the involvement of intra-trigeminal ganglionic communication in ectopic orofacial pain via nitric oxide (NO), nerve growth factor (NGF) or calcitonin gene-related peptide (CGRP) following orofacial inflammation or trigeminal nerve injury. Heat or mechanical hypersensitivity was induced in the ipsilateral whisker pad skin following inferior alveolar nerve transection (IANX) or lower lip inflammation, and suppressed by transient receptor potential vanilloid 1 (TRPV1) or $P2X_3$ receptor ($P2X_3R$) antagonism, respectively. Neuronal nitric oxide synthase (nNOS), NGF and CGRP expression in the trigeminal ganglion (TG) was increased following lower lip inflammation. Moreover, intra-trigeminal administration of tyrosine kinase receptor or nNOS inhibitor diminished the heat or mechanical hypersensitivity. The lower lip inflammation increased the number of $P2X_3R$- and TRPV1-positive TG neurons that innervate the whisker pad skin, which was annulled by anti-NGF intra-trigeminal ganglionic administration. The present findings suggest that intra-trigeminal ganglionic communication via NO, NGF or CGRP signaling resulted in upregulation and/or sensitization of TRPV1 or $P2X_3R$ in TG neurons following orofacial inflammation or trigeminal nerve injury, which may develop ectopic orofacial pain.

(COI: No)

S07-4

alpha-2/delta-1 subunit of dihydropyridine receptor in the trigeminal ganglion

Sato, Tadasu; Tachiya, Daisuke; Ichikawa, Hiroyuki (*Grad. Sch. Dent. Tohoku Univ., Sendai, Japan*)

Immunohistochemistry for alpha-2/delta-1 subunit of L-type calcium channel was performed on the rat trigeminal ganglion (TG). The immunoreactivity (IR) was detected in one third of TG neurons (32.8 %). These neurons were mostly small or medium-sized. A double immunofluorescence method revealed that half of alpha-2/delta-1-immunoreactive (IR) neurons were also immunoreactive for calcitonin gene-related peptide (54%). In addition, 41 % of alpha-2/delta-1-IR DRG and TG neurons contained vanilloid receptor subtype 1. However, co-expression of alpha-2/delta-1 with vanilloid receptor subtype 2 was infrequent (6%). A retrograde tracing method also demonstrated that alpha-2/delta-1-IR was common among cutaneous TG neurons (48.1 %) and relatively rare among tooth pulp TG neurons (24.4 %). Transection of the infraorbital nerve dramatically increased the number of alpha-2/delta-1 subunit-IR neurons in the TG. These findings indicated that small to medium-sized nociceptors with unmyelinated axons contain alpha-2/delta-1subunit of L-type calcium channel in the TG. The subunit in TG neurons may be associated with nociceptive transmission from oro-facial regions.

(COI: No)

S07-5

Vesicular Nucleotide Transporter (VNUT) regulates ATP signaling in Trigeminal Ganglion

Gunjigake, Kaori[1]; Goto, Tetsuya[2] ([1]*Kyushu Dental Univ., Kitakyushu, Japan;* [2]*Kagoshima Univ., Kagoshima, Japan*)

By oral nociceptive stimulation, neurons in trigeminal ganglion (TG) produce various neurotransmitters that communicate with other TG neurons. Though neurons in TG are surrounded by satellite glial cells (SGCs), little is known about the interactions between SGCs and TG neurons. We have focused on adenosine-5'-triphosphate (ATP) as a neurotransmitter, and investigated the association of the vesicular nucleotide transporter (VNUT); ATP transporter, with neurons and SGCs in TG using immunocytochemistry (IHC), reverse transcription polymerase chain reaction (RT-PCR), and in situ hybridization (ISH) in rats after rat upper molar extraction. After extraction, ATF3-immunoreactive (IR) neurons appeared in the maxillary nerve region. The ATF3-IR, damaged, neurons were surrounded by GFAP-IR, active, SGCs. Interestingly, ATF3 immunonegative neurons were also surrounded by GFAP-IR SGCs. The number of GFAP-IR SGCs and $P2X_3$ receptor-IR neurons was increased after extraction in a time-dependent manner. RT-PCR and in situ hybridization confirmed the increase of VNUT mRNA expression in neurons and SGCs in TG. Our results suggest that peripheral nerve injury induced the mutual activation of TG neuron and SGCs, possibly by VNUT-mediated ATP release.

(COI: No)

Symposium 8

Neural regulation of vascular function - Integration of anatomical and physiological evidence

(March 21, 14:00～15:30, Room E)

S08-1

Control of skin and skeletal muscle blood flow by sympathetic nerve activities in humans

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In humans, skin blood flow (SkBF) and sweat rate increase in hyperthermia; however, blood pooling in the cutaneous veins in an upright position and hypovolemia due to sweat loss decrease the venous return to the heart. If not compensated, they would threaten the maintenance of arterial pressure. To prevent this, cutaneous vasodilation is suppressed through baroreflexes; however, the efferent path has not been identified. Recently, we have reported that a component of skin sympathetic nerve activity (SSNA) synchronized with cardiac cycle might be involved in the mechanisms; however, the component might contain muscle sympathetic nerve activity (MANA). Here, we tested whether head-up tilt (HUT) would suppress the SSNA component while enhance MSNA in hyperthermia. In 12 men (22-24yr), wearing a perfusion suit, we measured right atrial volume (RAV), carotid artery diameter (CAD; echocardiography), esophageal temperature (T_{es}), cutaneous vascular conductance (CVC = SkBF/ mean arterial pressure), SSNA and MSNA (microneurography; the peroneal nerve; N=6) during supine and 30° HUT. SSNA component and CVC increased as T_{es} increased by 0.7℃ with 47-℃ water perfusion into the suit. We found that HUT suppressed the increases while enhanced MSNA and HR with reduced RAV and CAD. These results suggest that the SSNA component does not contain MSNA and both activities significantly contributes to the maintenance of arterial pressure by baroreflexes in hyperthermic humans in an upright position.

(COI: No)

S08-2

Neural vasodilator mechanisms contribute to increased blood flow to non-contracting muscle during one-legged cycling in humans

Matsukawa, Kanji; Ishii, Kei; Liang, Nan; Endo, Kana (*Dept Integrative Physiol, Grad Sch Biomed and Health Sci, Hiroshima Univ, Hiroshima, Japan*)

Whether neurally-mediated vasodilatation may contribute to exercise hyperemia has not been completely understood. Bülbring and Burn (1935) found for the first time the existence of sympathetic cholinergic nerve to skeletal muscle contributing to vasodilatation in animals. Blair et al. (1959) reported that atropine-sensitive vasodilatation in skeletal muscle appeared during mental stress in humans. However, such sympathetic vasodilator mechanism for muscle vascular bed in humans is generally denied at present, because surgical sympathectomy, autonomic blockade, and local anesthesia of sympathetic nerves cause no substantial influence on vasodilatation in muscle not only during mental stress but also during exercise. On the other hand, neural mechanisms may play an important role in regulating blood flow to non-contracting muscle. Relative changes in oxygenated-hemoglobin concentration (Oxy-Hb) of the contralateral vastus lateralis muscle, as index of tissue blood flow, were measured during 1-min one-legged cycling. The Oxy-Hb increased at the early period of exercise and the increase was sustained throughout exercise. Propranolol (0.1mg/kg iv) failed to affect the initial Oxy-Hb increase, whereas atropine (0.01-0.015mg/kg iv) abolished the initial increase. Both drugs blunted the later component of the Oxy-Hb increase during the exercise. Thus the rapid cholinergic and delayed β-adrenergic vasodilator mechanisms may contribute to increase muscle blood flow to non-contracting muscle during exercise.

(COI: No)

S08-3

Regulation mechanisms of blood flow examined by histochemistry

Kawamata, Seiichi; Kurose, Tomoyuki (*Inst.Biomed.HealthSci., Hiroshima Univ., Hiroshima, Japan*)

For better understanding of microvascular circulation, this study examined the proportion of open and functioning capillaries in the leg muscles (20, 30, 37 and 40℃), pancreas and small intestine of anesthetized rats. FITC-labeled *Lycopersicon esculentum* lectin was injected into the heart, mixed with blood and allowed to circulate for 3 min in the whole body. Open and functioning blood vessels were detected by immunostaining for this lectin bound to endothelial cells, whereas closed capillaries without blood flow were unstained. To detect all capillaries, sections were stained for PECAM-1 (CD31). The proportion of open and functioning capillaries in rat leg muscles was high in a period of 3 min at 37℃. Based on histochemical results, it was concluded that the blood flow of each capillary considerably decreased at 20 and 30℃ and probably increased at 40℃, whereas the proportion of open and functioning capillaries was essentially unchanged in the range of 20 to 40℃. In addition, the proportions of open and functioning capillaries are high and similar among the leg muscles, pancreas and small intestine in spite of their structural and functional differences. Therefore, microvascular systems of tissues and organs seem to control microvascular blood flow by changing the blood flow of each capillary, whereas the proportion of open and functioning capillaries is essentially unchanged. The blood flow considerably changes depending on the blood flow velocity and the size of blood vessels. These factors are probably regulated by the nerve activity, vasoactive substances, blood pressure and so on.

(COI: No)

S08-4

Neural control of pulmonary blood vessels in health and disease

Schwenke, Daryl O.[1]; Tsuchimochi, Hirotsugu[2]; Nagai, Hisashi[3]; Sonobe, Takashi[2]; Fujii, Yutaka[2]; Umetani, Keiji[4]; Shirai, Mikiyasu[2] (*[1]Dept of Physiol, University of Otago, Dunedin, New Zealand; [2]Dept of Cardiac Physiology, National Cerebral and Cardiovascular Research Institute, Osaka, Japan; [3]Dept of Forensic Medicine, University of Tokyo, Japan; [4]Japan Synchrotron Radiation Research Institute, Hyogo, Japan*)

Chronic intermittent hypoxia (IH) provokes a centrally-mediated increase in sympathetic nerve activity (SNA). The effect of this sympathetic hyper-excitation on the pulmonary vasculature remains unclear. We aimed to assess the effect of sympathetic excitation in modulating acute hypoxia pulmonary vasoconstriction (HPV), and the central β-adrenergic signalling pathway for facilitating the increase in SNA. Sprague-Dawley rats were exposed to IH for 8 hours/day for 6 weeks. Subsequently, pulmonary SNA was recorded in rats, and the pulmonary vasculature was visualized using microangiography. Pulmonary responses to acute hypoxia were assessed before and after central β-adrenergic receptor blockade (Metoprolol, 200 nmol). Chronic IH increased baseline SNA (110% increase), and exacerbated the sympathetic response to acute hypoxia. Moreover, the magnitude of HPV in IH-rats was blunted compared to control-rats (10% and 20% vasoconstriction, respectively). In only the IH rats, β-receptor blockade attenuated the hypoxia-induced increase in pSNA and exacerbated acute HPV, so that both sympathetic and HPV responses were similar to that of control-rats. These results provide compelling evidence that the centrally-mediated increase in SNA following IH acts to blunt the local pulmonary vasoconstrictor effect of acute hypoxia.

(COI: No)

S08-5

Neural control of cerebral cortical blood flow

Uchida, Sae (*Dept Auton Neurosci, Tokyo Metropol Inst Gerontol, Tokyo, Japan*)

The neural vasodilative system, consisting of cholinergic fibers projecting from the basal forebrain to the cerebral cortex, was found by Sato et al. in 1989. This finding has been confirmed by several other investigators and the underlying vasodilative mechanisms have been examined. At this symposium, our extensive research on the cholinergic vasodilative system will be introduced.

1. Decline of the cholinergic vasodilative system in very old rats and the underlying mechanism

The cholinergic cortical vasodilation in response to stimulation of nucleus basalis of Meynert (NBM) is maintained in old rats (2 years old), but it declines in very old rats (3 years old). The decline of the vasodilation in very old rats is mainly due to age-related decline of nicotinic acetylcholine receptor (nAChR) activity. The subtype of nAChRs that mediates the cortical vasodilation is $\alpha 4\beta 2$, but not $\alpha 7$.

2. Effect of acupuncture on the cholinergic vasodilative system and their relationship with aging

Acupuncture-like stimulation of a forepaw, the back, and a hindpaw increases cortical ACh release. Cortical blood flow (CBF) is increased by the stimulation of paws but it is not affected by stimulation of the back. The stimulation of paws increases blood pressure (BP), whereas that of the back decreases BP. Thus, an increase in ACh release is independent of BP changes, whereas responses in CBF are influenced by BP changes. In a rat model, where BP is not changed by forepaw stimulation, an increase in CBF is still elicited via the cholinergic vasodilative system. Acupuncture-induced activation of the cholinergic vasodilative system is observed even in very old rats.

(COI: No)

Symposium 9

Regulatory mechanisms of sperm properties toward fertilization success

(March 21, 14:00～15:30, Room F)

S09-1

ADAM3 and sperm fertilizing ability

Ikawa, Masahito (*Res Inst Micobial Dis, Osaka Univ, Osaka, Japan*)

Sperm binding to zona pellucida (ZP) has been believed to be prerequisite for the physiological acrosome reaction and the subsequent fertilization process in mammals. In 1997, we reported that the testis specific endoplasmic reticulum chaperone, calmegin (Clgn), is required for sperm binding ability to ZP1. To date, more than 10 knockout mouse lines (Ace, Adam1a, Adam2, Adam3, Calr3, Clgn, Pdilt, Pmis2, Rnase10, Tex101, Tpst2) were reported to be infertile and share the similar phenotype. Strikingly, all the mutant mice showed the defective ADAM3 localization in the mature spermatozoa. Thus sperm ADAM3 was considered to play indispensable role during fertilization by mediating sperm-ZP binding. However we showed that the mutant spermatozoa lacking ADAM3 successfully fertilize ZP intact eggs if they were surrounded in cumulus cells2. The data suggest that the presence of numerous acrosome intact sperm binding to ZP surface of cumulus free eggs is less important than previously supposed. The idea is supported by the report that most fertilizing mouse spermatozoa begin their acrosome reaction during cumulus penetration before contact with the ZP3. More importantly, we found that the spermatozoa lacking ADAM3 are unable to migrate through utero-tubal junction (UTJ) in the female reproductive tract. Therefore the mechanism of sperm migration through UTJ should be investigated in the future study.

(COI: No)

S09-2

The non-genomic regulation of sperm hyperactivation by steroids

Fujinoki, Masakatsu (*Dept Physiol, Sch Med, Dokkyo Med Univ, Mibu, Tochigi, Japan*)

During capacitation, mammalian spermatozoa are hyperactivated. Hyperactivation is a modification of flagellar movement to create the driving force for penetrating the zona pellucida. Recently, it has been suggested that several hormones regulated sperm hyperactivation. In hamster, progesterone enhances sperm hyperactivation, and estradiol suppresses progesterone-enhanced hyperactivation. Both steroids dose-dependently affect sperm hyperactivation. When progesterone enhances sperm hyperactivation, progesterone binds to membrane progesterone receptor and activates phospholipase C and protein kinases. Finally, tyrosine phosphorylations of sperm proteins are increased and/or enhanced together with enhancement of sperm hyperactivation. Estradiol also binds to membrane estrogen receptor when estradiol suppresses progesterone-enhanced hyperactivation. Although detailed signals are not clear, many tyrosine phosphorylations of sperm proteins are inhibited by estradiol together with suppression of progesterone-enhanced hyperactivation. Interestingly, regulation of sperm hyperactivation by steroids was disrupted by diethylstilbestrol.

(COI: No)

S09-3

Ever-changing fertilization paradigms-in a case of mammalian sperm acrosome reaction

Hirohashi, Noritaka (*Oki Marine Station, Shimane Univ., Japan*)

Science is interesting because search for truth never ends. The sperm acrosome reaction (AR), first discovered by Jane Clark Dan in echinoderms, is believed to be essential for sperm-egg fusion to occur in the protostomes and the Deuterostomes. In eutherian mammals, the AR is thought to be prerequisite for the sperm to pass through the extracellular investment of the oocyte as referred to the zona pellucida (ZP). A long-standing hypothesis in the commitment of the AR states that the ZP induces the AR. This has been reinforced by the following empirical data using acid-solubilized ZP glycoproteins in different mammalian species. Research moved into molecular details and the technology also moved from biochemistry to genetic engineering. The "green" sperm was made in 1999 and the dogma has been challenged since then. I shall present the current status of the mouse AR with a fascinating imaging tool.

(COI: No)

S09-4

Regulation of fertilization competence of the egg-coating envelope by the interaction between *Xenopus* dicalcin and gp41

Miwa, Naofumi[1]; Ogawa, Motoyuki[2]; Hanaue, Mayu[1]; Takamatsu, Ken[1]
([1]*Dept Physiol, Sch Med, Toho Univ, Tokyo, Japan;* [2]*Dept Med Learn, Sch Med, KItasato Univ, Kanagawa, Japan*)

Mature oocytes of animals are surrounded by an extracellular egg-coating envelope (called vitelline envelope in frogs, VE) that plays important roles in the processes of fertilization and thereafter, up to implantation of fertilized egg. Evidence from lectin staining of oocytes has pointed out divergent structural patterns among the filamentous egg-coats of unfertilized eggs, but yet no study has shown precise structural basis on varied fertility. We previously found that *Xenopus* dicalcin, present in the VE, constitutively suppresses the efficiency of fertilization through binding to gp41, a glycoprotein of the egg envelope. By using synthetic peptides that correspond to amino-acid regions responsible for the interaction between two proteins, we clamped the VE either in fertilization-competent or -incompetent status, and investigated its nanoscale structure. Our electron microscopy analyses revealed a disorganized filamentous meshwork within the competent VE, but a well-organized meshwork was observed in the incompetent one. *In vivo* lectin-staining pattern of the VE also revealed remarkable differences between two VE statuses. These results demonstrated the structural basis on the fertilization competence of the egg-coats and observed transition of the fertilization competence by extrinsic treatment promotes the development of efficacious drugs for contraceptive strategy and the treatment of infertility in animals. Molecular insights of fertilization competence will also be discussed.

(COI: No)

S09-5

Equatorin-mediated sperm-egg interaction

Toshimori, Kiyotaka; Ito, Chizuru; Yamatoya, Kenji (*Grad.Sch.Med.Chiba Univ., Chiba, Japan*)

Mammalian fertilization is consisted of multistep processes mediated by sperm and egg factors. The molecular mechanism controlling gamete interaction requires mediation by gamete factors but remains unsolved. Now it is widely accepted that sperm Izumo1 and egg Cd9 are essential for gamete fusion. However, since no fusogenic domain is detected in Izumo1 and Cd9 and no direct interaction between them is reported, additional factors are expected. Recently Juno and Cd9 are reported as egg Izumo1 receptor and Juno partner, respectively. We first reported equatorin as a MN9 antigen against a monoclonal antibody MN9 in the mouse and human sperm. Equatorin is registered as a sperm protein (EQTN) encoded by Eqtn (EQTN). Inhibition assays with MN9 reduce the fertility without inhibition of sperm penetration into zona pellucida (ZP) but inhibit sperm-egg interaction and egg activation. Equatorin is type 1 membrane protein with single transmembrane domain and short cytoplasmic tail. High resolution fluorescence microscopy using transgenic B6-Tg(Eqtn-EGFP) male mice and immunogold electron microscopy with MN9 revealed that equatorin is Golgi-derived and integrated into the acrosomal membrane in spermatids and then relocated on the equatorial segment during the acrosome reaction. B6-Tg(Eqtn-EGFP) distribution pattern at the equatorial segment was uniform before penetration on the ZP, but much perturbed, reducing the staining intensity, after penetration through ZP. This change is presumed to correlate for sperm to gain the competency to fuse with oolemma. The function is currently underway using Eqtn-deficient male mice.

(COI: No)

Symposium 10

Forefront of exo- and endocytosis research

(March 21, 14:00～15:30, Room H)

S10-1

Visualizing exocytosis of single synaptic vesicles at the calyx-type presynaptic terminal

Midorikawa, Mitsuharu; Sakaba, Takeshi (*Grad Sch Brain Sci, Doshisha Univ, Kyoto, Japan*)

To support fast and reliable synaptic transmission at central nerve system, presynaptic terminals need to organize exocytosis precisely. However, the fate of each synaptic vesicle inside presynaptic terminals remains largely unknown. Applying TIRF microscopy to the presynaptic terminal of rat calyx of Held synapse at the brainstem, we visualized synaptic vesicles near the plasma membrane, and sites of calcium influx.The presynaptic terminal of a rat calyx of Held was acutely dissociated. Synaptic vesicles were labeled with FM dye. The presynaptic terminal was whole cell voltage-clamped, and was stimulated by depolarizing pulses. The sites of calcium influx were visualized using fluorescent calcium indicator, which was loaded into the terminal through a recording patch pipette.Upon stimulation, a fraction of FM-labeled vesicles underwent fusion, which could be identified as the release of FM-dye from the vesicles. We also found docking of synaptic vesicles and examined the distribution of these events against the time of stimulation. When calcium indicator was loaded into the cell, the sites of calcium entry could be defined as sites where the amplitude of fluorescence increase was large. We visualized vesicles and calcium entry sites from the same cell by applying two-color TIRF imaging, and examined the spatio-temporal relationship between those two sites.

(COI: No)

S10-2

2-photon FLIM analysis of SNARE assembly in neuron and endocrine cells

Takahashi, Noriko[1]; Sawada, Wakako[1]; Watanabe, Satoshi[1,2]; Noguchi, Jun[1]; Ohno, Mitsuyo[1]; Kasai, Haruo[1] ([1]*Dept Struct Physiol, Grad Sch med, Univ Tokyo, Tokyo, Japan;* [2]*Dept Bioengineering and Robotics, Grad Sch Engineering, Tohoku Univ, Sendai, Japan*)

We have investigated the SNARE assembly at the presynaptic terminals of neurons and plasma membranes of pancreatic beta cells, using two-photon fluorescence lifetime imaging (2p-FLIM) of Forster resonance energy transfer (FRET). We constructed the FRET probes of SNAREs, VAMP2 and syntaxin1 and SNAP25, by labeling with Turquoise (donor) or Venus (acceptor). In pancreatic beta cells, most SNAREs were unassembled in the plasma membranes, except for a small fraction (6%) of SNAP25 forming a binary complex with syntaxin. In contrast, a significant fraction (20%) of syntaxin formed the ternary trans-SNARE complexes in the boutons. Most trans-SNAREs were converted to cis-SNARE after exocytosis, and they were rapidly spread along axons by diffusion. The FRET ratio was correlated with the post-synaptic spine sizes and release probability. Thus, the SNARE configurations are diverse and regulated to allow the ultrafast exocytosis in the active zone, while slow exocytosis in the islet beta cells. There is huge diversity in resting SNARE complexes, and our FRET/2pFLIM enabled imaging of fusion readiness in intact live or chemically fixed secretory preparations.

(COI: No)

S10-3

Comprehensive functional analysis of Rab family small GTPases in dense-core vesicle exocytosis

Fukuda, Mitsunori (*Lab Membr Trafficking Mech, Grad Sch Life Sci, Tohoku Univ, Sendai, Japan*)

Rab-type small GTPases are key players in membrane trafficking, which underlies a variety of cellular events, including regualted secretion from secretory cells. Rabs function as switch molecules that cycle between two nucleotide-bound states, a GTP-bound active state and a GDP-bound inactive state, and the active Rabs drive various steps or types of membrane trafficking by recruiting their specific effector molecules. In mammals, more than 60 Rab isoforms have been reported, but because of their large numbers the precise functions of most mammalian Rabs remain largely unknown. To comprehensively analyze mammalian Rab isoforms, we have recently developed new tools, named "Rab panels", which include collections of Rab expression plasmids, siRNAs, and antibodies. By using these tools, we systematically screened for Rabs that are involved in dense-core vesicle exocytosis in neuroendocrine PC12 cells and succeeded in identifying three Rabs, Rab3A, Rab27A, and Rab33A, as dense-core vesicle-resident Rabs. We showed by TIRFM that two closely related isoforms, Rab3A and Rab27A, regulate the docking step of dense-core vesicles through interaction with their shared effectors, e.g., Slp4-a and rabphilin. By contrast, Rab33A is involved in both basal and regulated hormone secretion likely through interaction with an autophagic protein Atg16L1. We also applied these tools to other types of secreting cells and identified several cell-type specific secretory Rabs. Based on these results, we discuss the diversity and uniformity of Rab proteins in the regulation of various secretion events.

(COI: No)

S10-4

Study of Secretory Pathway Using Optogenetics

Nakata, Takao (*TMDU, Tokyo, Japan*)

Optogenetics is considered to be a study of neural circuit by regulating certain populationof neurons by channelrodopsin and light in vivo. We use optogenetics to study subcellular mechanism of cell signaling. Today we introduce our development of optogenetic tool and application to the study of secretory pathway in cell level.Ca is a champion of signaling molecules, in its diversity of the targets, number of researchers. In synaptic transmission, its speed is msec order, the vesicle size was small(50nm). Howeever, there are many other phenomenon slower. Endocrine cells such as insulin secreting cells, Ca was the 2nd messenger, and secretory vesicles are bigger. We made a tool which regulalte Ca concentration by light.Similar attempt was reported in USA but, it appeared to be too slow as a tool. We show the characterization of the switch and report on its application in cell biology.

(COI: No)

S10-5

Imaging of individual endocytosis of AMPA receptor around postsynaptic membrane

Hirano, Tomoo (*Dept Biophys, Grad Sch Sci, Kyoto Univ, Kyoto, Japan*)

AMPA-type glutamate receptors (AMPARs) dynamically change during synaptic plasticity. In the hippocampal long-term depression (LTD), the number of AMPARs decreases at the postsynaptic membrane. During LTD endocytosis of AMPAR takes place. However, when and where each subtype of AMPAR is endocytosed remains unclear. To address this question, we have developed an experimental method to visualize individual endocytosis of AMPAR with a high signal to noise ratio. We coated a glass coverslip with Neurexin, a cell adhesion molecule involved in synapse formation, and cultured hippocampal neurons on the coverslip. This procedure induced formation of postsynaptic-like membrane (PSLM) on the glass surface. Then, AMPAR whose extracellular domain is tagged with pH-sensitive fluorescent protein SEP is expressed in a neuron, and observed with total internal reflection microscopy. SEP is fluorescent at neutral pH, but not at low pH such as in the endosome. After endocytosis of SEP-AMPAR the fluorescent signal decreases as pH of intracellular vesicle gets lower, but it takes several seconds. We changed pH of extracellular solution quickly and repeatedly using U-tube. When pH of external solution is 6, the fluorescent signal from SEP-AMPAR on the cell-surface becomes undetectable, and only the fluorescent signal from SEP-AMPAR endocytosed during several seconds before the pH change to 6 was detected. We observed individual endocytosis of SEP-AMPAR both in the vicinity of PSLM and in the extra-synaptic membrane. Furthermore, a LTD-inducing chemical stimulation increased the frequency of individual endocytosis of SEP-AMPAR.

(COI: No)

Symposium 11

Expression, Structure and Function of Thermosensitive TRP channels

(March 21, 14:00～15:30, Room I)

S11-1

Thermosensitive TRP channel contributes to oral membrane protection

Kido, Mizuho A (*Dept.Molecular Cell Biology, Grad.Sch.Dent.Kyushu Univ., Fukuoka, Japan*)

The oral cavity provides an entrance to the alimentary tract, for which it serves as a protective barrier against environmental stimuli. The oral mucosa sense dynamic changes in the oral cavity; they are required to detect changes in the external environment and then the epithelia adapts to the environment structurally because of its location. However, the molecular basis of oral epithelial maintenance in response to the changes in the oral cavity is still not clear. We explored the expression of transient receptor potential (TRP) channels in oral epithelia and found the most abundant expression of TRPV3 and TRPV4 among thermosensitive TRP channels observed. Through calcium imaging and patch-clamp analyses, we confirmed that the oral epithelial cells express functional TRPV3 and TRPV4 with thermosensitive properties. Since we found more delayed wound healing after tooth extraction in TRPV3 gene deleted mice than in wild type mice, TRPV3 suggested a contribution to wound healing via EGFR signaling. Cell-cell adhesion is also important for epithelial integrity. We found impaired cell- cell contact in TRPV4 gene deleted mice, suggesting that TRPV4 contribute to the epithelial barrier in the oral membrane. Warm temperatures activated oral epithelia via TRPV3 and TRPV4, which suggested they play an important role in the epithelial barrier and its maintenance.

(COI: No)

S11-2

Temperature elevation in epileptogenic zone promotes epileptic events through TRPV4 activation

Shibasaki, Koji (*Dept. of Mol.Cell. Neurobiology, Gunma Univ. Grad. Sch.of Med., Maebashi, Japan*)

Physiological brain temperature is an important determinant for neuronal functions, and it is well established that changes in temperature have dynamic influences on brain neuronal excitabilities. We have clearly revealed that a thermo-sensor TRPV4 (activated above 34℃) is activated by physiological temperature in hippocampal neurons and thereby controls their excitability. Therefore, if local brain temperature could dynamically elevate depending on the neuronal activities, a thermo-sensor TRPV4 can enhance electrical excitability in neurons, and might lead to hyperexcitability. In this study, we focused on epilepsy, since it was caused by hyperexcitability of neurons. We generated a model of partial epilepsy by utilizing kindling stimuli in ventral hippocampus of wild type (WT) or TRPV4KO mice, and measured electroencephalogram (EEG). The frequencies of epileptic EEG in WT mice were significantly larger than those in TRPV4KO mice. These results strongly indicate that TRPV4 activation is involved in disease progression of epilepsy. We expected that the disease progression enhanced hyperexcitability, and lead to hyperthermia in the epileptogenic zones. To confirm it, we developed a new device to measure exact brain temperature only in restricted local area. From the recording results by the new device, we revealed that the brain temperatures in epileptogenic zones were dramatically elevated compared with normal regions. Furthermore, we demonstrated that the temperature elevation was critical for disease progression.

(COI: No)

S11-3

Roles of redox-sensitive TRPA1 in painful peripheral neuropathy induced by chemotherapy

Nakagawa, Takayuki[1,2]; Kaneko, Shuji[2] ([1]*Dept Clin Pharmacol & Ther, Kyoto Univ Hospital, Kyoto, Japan;* [2]*Dept Mol Pharmacol, Grad Sch Pharmaceu Sci, Kyoto Univ, Kyoto, Japan*)

Peripheral neuropathy is a common side effect of chemotherapeutics. Notably, oxaliplatin (L-OHP), a platinum-based agent, causes peculiar acute peripheral neuropathy, which appear in almost all patients during or within hours after infusion, and is triggered or exacerbated by cold, but the mechanisms are poorly understood. In this study, we examined the roles of redox-sensitive TRPA1 in L-OHP-induced acute peripheral neuropathy in mice. An i.p. administration of L-OHP or its metabolite oxalate induced cold hypersensitivity within 2 h, which was abolished by a TRPA1 antagonist or deficiency. TRPA1 agonist-evoked nocifensive behaviors were significantly enhanced in mice pretreated with L-OHP. Pretreatment of cultured mouse DRG neurons with L-OHP for 1-4 h increased the responsiveness of TRPA1, but not TRPM8 and TRPV1. In hTRPA1/HEK293 cells, high concentrations of L-OHP evoked a Ca^{2+} response and increased whole-cell currents, which were mediated through ROS production. On the other hand, pretreatment with relatively low concentrations of L-OHP for 2 h enhanced H2O2-evoked Ca^{2+} response and currents in hTRPA1/HEK293 cells. Furthermore, the L-OHP-enhanced responsiveness of TRPA1 was inhibited by co-expression of mutated proline hydroxylase (PHD)1-3 or disappeared in mutated TRPA1 of Pro394, a residue hydroxylated by PHDs. These results suggest L-OHP could sensitize TRPA1 function via dehydroxylation of Pro394 in TRPA1 N-terminal by inhibition of PHD activity, and the sensitized TRPA1 is activated by ROS production.

(COI: No)

S11-4

Modulatory mechanisms of TRP channels TRPA1/V1

Noguchi, Koichi (*Hyogo College of Medicine, Nishinomiya, Hyogo, Japan*)

It is well suggested that TRPA1 is an important component of the transduction machinery through which noxious irritants and endogenous proalgesic molecules depolarize nociceptors to elicit pain, in addition to the established effect of TRPV1 on noxious heat transduction. The regulatory mechanisms of TRPA1/V1 in persistent pain collect much attention, and we previously reported a short-term treatment of artemin, a GDNF family member, significantly suppressed the AITC-induced TRPA1 currents using a whole-cell patch clamp analysis, and suggested a rapid and inhibitory role of artemin in regulation of sensory neurons. Here, we examined the long-term effect of artemin on the gene expression. We found that artemin increases locally in skin over long periods of time after peripheral inflammation, and the synthesis of artemin increased at a site distal to the nerve injury. In vivo repeated artemin injections into the periphery increased the gene expression of TRPA1/V1 in DRG, and also induced mechanical and heat hyperalgesia. All data indicate the positive regulatory role of periphery-derived artemin on the TRPV1/A1 expression in DRG neurons in pathological conditions such as inflammatory and neuropathic pain. Next, we examined another inhibitory modulation of TRPA1/V1 by the resveratrol, which is widely contained in natural food and historical medicines. We found that resveratrol dose-dependently suppressed the AITC-induced currents in HEK cells that express TRPA1, as well as in rat DRG neurons. In conjunction with behavioral data, we could suggest that resveratrol may have an inhibitory effect on TRP channels.

(COI: No)

S11-5

A pain-enhancing mechanism through the interaction between TRPV1 and anoctamin

Tominaga, Makoto (*Div Cell Signaling, Okazaki Inst Integrative Bioscience, Okazaki, Japan*)

Capsaicin receptor TRPV1 is activated by various noxious stimuli, and converted the stimuli into electrical signals in primary sensory neurons. It is believed that cation influx through TRPV1 causes depolarization, leading to the activation of voltage-gated sodium channels, followed by action potential generation. We report that the apparent capsaicin-evoked firing is induced by two components: a cation influx-mediated depolarization due to TRPV1 activation and a subsequent anion efflux-mediated depolarization via activation of anoctamin 1 (ANO1), a calcium-activated chloride channel, due to the entry of calcium through TRPV1. This interaction between TRPV1 and ANO1 is based on the physical binding of the two proteins. Capsaicin activated chloride currents in an extracellular calcium-dependent manner in HEK293T cells expressing TRPV1 and ANO1, and capsaicin-evoked inward currents were significantly inhibited by a specific ANO1 antagonist, T16Ainh-A01 (A01) in mouse DRG neurons. In addition, capsaicin-evoked action potential generation was drastically inhibited by A01. Furthermore, pain-related behaviors in mice treated with capsaicin were significantly reduced by the concomitant administration of A01. These results indicate that the TRPV1-ANO1 interaction is a significant pain-enhancing mechanism in the peripheral nervous system. Therefore, the TRPV1-ANO1 interaction would be a promising target for the development of novel analgesic agents.

(COI: No)

Symposium 12

Frontier of the structural and functional investigation of the kidney

(March 21, 14:00～15:30, Room J)

S12-1

Ciliary subdomains and abnormality in the kidney of inv mutant mice

Yokoyama, Takahiko[1]; Tsuji, Takuma[1,2] ([1]*Grad. Sch. Med. KPUM, Kyoto, Japan;* [2]*Grad. Sch. Med. Nagoya univ. Nagoya, Japan*)

Primary cilia in the kidney are a hair like structure projecting from the surface of nearly all cells. Inside of the cilia is the axoneme that is consisting of 9 circumferentially arranged microtubule doublets. The cilia are structurally divided longitudinally into sub-compartments that include the rootlet, the basal body, the transitional zone, the ciliary shaft and the tip. Nephronophthisis is a most common genetic disease that causes the end-stage renal failure. Sixteen causative genes have been identified, and all examined products are localized in the primary cilia and/or basal body. The INV compartment is a proximal region of the ciliary shaft in which inv/nephrocystin2 is localized.In the presentation, we show that the doublet of the axoneme is surprisingly short in renal cilia and that the region is likely to corresponding to the Inv compartment. The length of the microtubule doublet region is not altered in the inv mutant. However, in the inv mutants, the ciliary rootlets are not well developed and singlet microtubules of the axoneme are turbulent.

(COI: No)

S12-2

Role of calcium sensing receptor (CaSR) in type-B intercalated cell of mouse kidney collecting duct during acid/base and Ca salts-loadings

Yasuoka, Yukiko[1]; Kawahara, Katsumasa[1]; Sato, Yuichi[2]; Nonoguchi, Hiroshi[3]
([1]*Dept of Physiology, Kitasato U. Sch. of Med, Sagamihara, Kanagawa, Japan;* [2]*Dept of Mol. Diagnostics, Kitasato U. Sch. of Allied Health Sci, Sagamihara, Kanagawa, Japan;* [3]*Internal Med., Kitasato U. Medical Center, Kitamoto, Japan*)

It is believed that hypercalciuria stimulates the urinary acid excretion in type-A intercalated cell (IC-A) and inhibits luminal water permeability in principal cell (PC) to prevent urolithiasis through activation of apical calcium-sensing receptors (CaSR) in collecting ducts (CD). However, we found that CaSR, genetically same as RaKCaR in TAL, only localized in the basolateral membrane of type-B intercalated cell (IC-B), not at PC and IC-A through the CD by using a high sensitive in situ hybridization technique and immunohistochemistry (Yasuoka et al. 2014). The levels of CaSR mRNA and protein expression in IC-B were increased and decreased, respectively, during alkali- and acid-loading. The CaSR of IC-B may contribute to alkali excretion. On the other hand, neutral high calcium ($CaCO_3$ + Ca phosphate) loading for 28 days decreased urine pH, and the expression levels of H^+-ATPase, AE1 mRNA in IC-A and Pendrin, CaSR mRNA in IC-B increased co-operatively. High Ca diet (neutral CaP/CaC salts) promotes co-operative activation of IC-A and IC-B for increasing urinary acid and alkali excretion. The basolateral CaSR of IC-B may maintain plasma acid-base balance in accordance with preventing urolithiasis during high Ca diet.

(COI: No)

S12-3

Regulation of podocyte structure and function: roles of Rho family proteins and their modulators

Nagase, Miki; Sakai, Tatsuo (*Grad.Sch.Med.Juntendo Univ., Tokyo, Japan*)

Rac1, a member of the Rho-family small GTPases, regulates diverse cellular functions, including organization of the actin cytoskeleton (formation of lamellipodia and membrane ruffles), cell adhesion and motility, generation of reactive oxygen species, and gene transcription. Recent studies implicate a role of Rac1 overactivation in podocyte injury and glomerulosclerosis. Rac1 activity is enhanced in podocytes by proteinuric stimuli such as angiotensin II, puromycin aminonucleoside, lipopolysaccharide, diabetic condition, and HIV infection, causing motile phenotype and foot process effacement. We reported that mice deficient in RhoGDI α, a negative regulator of Rac1, resulted in Rac1 overactivation in the kidney, and spontaneously developed Rac1-dependent podocyte injury and focal glomerulosclerosis. In these mice, Rac1 potentiated the activity of mineralocorticoid receptor (MR) in a ligand-independent manner, thereby accelerating podocyte injury. We subsequently demonstrated the crosstalk of Rac1 and MR pathways in several kidney injury models. Podocyte-specific RhoGDI α knockout mice exerted podocyte injury similar to systemic knockout mice. Again, MR antagonist perfectly ameliorated the injury. On the other hand, podocyte-specific Rac1 depletion lead to podocytopathy and glomerulosclerosis by a different mechanism, because MR signaling was suppressed and MR antagonist was ineffective in these mice. These findings suggest that proper level of Rac1 activity is essential for the morphological and functional integrity of podocytes.

(COI: No)

S12-4

The role of podocyte injury in progression to glomerulosclerosis

Asanuma, Katsuhiko (*Kyoto Univ. Grad. Sch. Med., Kyoto, Japan*)

Podocytes, are highly specialized glomerular visceral epithelial cells that cover the outer layer of the glomerular basement membrane (GBM). Based on their cytoarchitecture, podocytes may be divided into three structurally and functionally different segments: cell body, major processes, and foot processes (FPs) that attach to the underlying GBM. The FPs of neighboring podocytes regularly interdigitate, leaving between them filtration slits that are bridged by an extracellular structure, known as slit diaphragm (SD). Therefore, podocytes form the final barrier to protein loss, which explains why podocyte injury is typically associated with marked proteinuria. Chronic podocyte injury may cause detachment from the GBM, resulting in depletion. FP effacement represents the most characteristic change in cell shape of injured podocytes. FP effacement depends on the disruption of both the actin cytoskeletal network, and the SD in the podocytes. This symposium highlights some of our recent findings for translating podocyte biology into new therapies and examinations of podocyte injury.

(COI: No)

Symposium 13

Space Medicine I : Living with Gravity

(March 21, 15:30～17:00 Room B)

S13-1

Skeletal muscle plasticity in response to gravitational loading

Goto, Katsumasa (*Dept Physiol, Grad Sch Health Sci, Toyohashi SOZO Univ, Aichi, Japan*)

Human skeletal muscle system has evolved by adapting to the environment of a gravity. Therefore, the functional and structural properties of skeletal muscles, especially antigravitational soleus muscle, change in response to gravitational level. For example, overloading causes to increase in skeletal muscle mass, so-called muscle hypertrophy. On the other hand, skeletal muscle atrophy is induced by unloading as well as aging. It is well known that these changes in skeletal muscle mass are reversible. Although skeletal muscle exhibits a large plasticity in response to gravitational levels, the molecular mechanism(s) for loading-dependent adaptation of skeletal muscle is not fully elucidated. It has been generally accepted that muscle satellite cells, a skeletal muscle-specific stem cell, play a crucial role in skeletal muscle plasticity, especially the regeneration of injured skeletal muscle cells. Muscle satellite cells are activated by muscle injury, and consequently proliferate and fuse to injured muscle cells or form a new muscle fiber. However, unloading prevent the proliferation of muscle satellite cells in injured skeletal muscle. The observations suggest that muscle satellite cells have a sensitivity to gravitational level. This study was supported, in part, by Grants-in-Aid for Scientific Research from JSPS, Grants-in-Aid for Challenging Exploratory Research from JSPS, The Uehara Memorial Foundation, and The Naito Foundation.

(COI: No)

S13-2

Unique epigenetic properties in anti-gravitational muscle

Kawano, Fuminori (*MSPA, Grad Sch Med, Osaka Univ, Japan*)

Slow skeletal muscle, such as soleus, shows a tonic neural activity to maintain the posture against gravity, so-called anti-gravitational muscle. Acquisition of the characteristics of slow muscles is resulted from such muscular activity during the growing period. It is well known that loss of the gravitational load causes a shift of muscle properties toward faster phenotype and metabolism, as well as the sever atrophy. However, it is unknown what is an essential factor accounted for the ontogeny of slow muscle characters. Histone modification, known as one of the epigenetic regulations of gene transcription, plays a critical role for the transcriptional onset via the conformational change of chromatins. We have identified the major differences of histone modification between slow- and fast-twitch skeletal muscles by using ChIP-seq. Generally, it is known that transcriptionally active histone modification, H3K4me3 and H3 acetylation, maps near the transcription start site. In fast-twitch plantaris muscle of rat, this typical pattern was noted in the loci of activated genes. However, genome-wide analysis by ChIP-seq also revealed that no relationship was observed between the expression of specific genes and active histone marks in slow-twitch soleus muscle. We also found that the up-regulation of slow genes in plantaris muscle, which are related to enhanced muscular activity, were not associated with additional active modifications. These findings indicate that the anti-gravitational, slow-twitch, muscle has a unique set of histone modification, may be due to the muscular activity present under gravity.

(COI: No)

S13-3

Effects of hypergravity on coordinated left and right arm movements

Wada, Yoshiro (*Dept Otolaryngology, Nara Med, Univ, Kashihara, Japan*)

We can move left and right arms symmetrically even with no visual feedback under various conditions because the brain generates appropriate motor commands to each arm. To examine the contribution of sensory inputs and the central nervous system (CNS) to these coordinated arm movements, the horizontal symmetric reaching arm movements (finger-finger docking tasks) were conducted with/without a weight on one arm (0.5 kg and 1.0 kg) under one- and two-gravity conditions in six normal subjects. The main results were: 1) the arm with a weight went down compared to the control arm under one-gravity condition; 2) on the contrary, the arm with a weight went up compared to the control arm under two-gravity condition. Those observations cannot be explained by somatosensory inputs. We will discuss the contribution of otolith inputs and/or the CNS to those results based on data from additional experiments.

(COI: No)

S13-4

Influence of the gravitational acceleration on body balance and gaze stability during walking

Hirasaki, Eishi (*Primate Res. Inst. Kyoto Univ., Inuayama, Japan*)

We have been studying body, head and eye movements during walking gait to understand the strategies for controlling posture and body balance during locomotion. Our results revealed that the coordinated motions of the eyes and head play important roles in maintenance of gaze stability. For example, when the humans walk, the body moves up and down, according to the reciprocal motions of the legs. These movements, which disturb clear vision, are partly compensated by head pitch rotation. The facts that the compensatory head rotations are deteriorated in the patients with vestibular disfunctions and in the postflight astronauts suggest the idea that it is induced by the vestibular signals via the linear vestibulocollic reflex. As a consequence of the compensatory head rotation, lines representing the naso-occipital axis of the head intersect at approximately a common point during walking, which is referred to as the "Head fixation point". It is constantly located approximately 1m in front of the head, and provides a stable platform on which the angular vestibuloocular reflex does the rest of the work to maintain gaze. During curved walking, things are more complicated due to visual flow and tilt of the gravito-inertial axis (GIA). The former induces the eye nystagmus, but coordinated motions of head yaw and slow phase of eye movements intermittently stabilize gaze in yaw plane. In coronal plane, the head tilts in the same direction of tilts of GIA. The head tilt increases as walking speed increases, independently from body tilt, suggesting that the head tilt is induced by the tilt of GIA via otolith input.

(COI: No)

S13-5

Variation of orthostatic arterial pressure response related to vestibular function

Tanaka, Kunihiko[1]; Nakamura, Koji[2]; Abe, Chikara[3]; Morita, Hironobu[3] ([1]*Dept Radiol Tech, Gifu Univ Med Sci, Seki, Japan;* [2]*Dept Med Tech, Gifu Univ Med Sci, Seki, Japan;* [3]*Dept Physiol, Grad Sch Med, Gifu Univ, Gifu, Japan*)

The vestibular system contributes to determination of the body orientation with respect to gravity. We clarified that maintenance of mean arterial pressure (MAP) at the onset of head-up tilt (HUT) is controlled by the vestibular system. However, changes in MAP at the onset of HUT vary among each subject. Some subjects show increase in MAP (UP), compared with that during supine position, but the others show decrease (DOWN). In healthy subjects, balance between the left and right otolith function is involved in the difference. To investigate the vestibular function for autonomic control of circulatory system in detail, heart rate variability (HRV) was measured with applying galvanic vestibular stimulation (GVS), which stimulates vestibular nerves from both semicircular canals and otolith organs. Changes in high frequency component of HRV (HF), an index of parasympathetic nerve activity, was inversely correlated with changes in MAP at the onset of HUT. Thus, parasympathetic nerve activity might be relatively higher in DOWN subjects at the onset of HUT, compared with that in UP subjects. Furthermore, changes in HF were also correlated with the changes in MAP with applying sound pressure, which stimulates only otolith organs. Those observations suggest presence of vestibulo-parasympathetic reflex, and the reflex is involved in variability of changes in MAP at the onset of HUT.

(COI: No)

Symposium 14

Sensory and motor mechanisms regulating feeding behavior

(March 21, 15:30～17:00, Room D)

S14-1

The roles of oral-brain-gut interaction in detection, transmission and modulation of taste signals and regulation of food intake

Ninomiya, Yuzo[1,2]; Takai, Shingo[1]; Yoshida, Ryusuke[1]; Shigemura, Noriatsu[1] (*[1]Sect Oral Neurosci, Grad Sch Dental Sci, Kyushu Univ, Fukuoka, Japan; [2]Div Sens Physiol, Res Dev Center for Taste and Odor Sensing, Kyushu Univ*)

In the brain, leptin reduces food intake by acting on hypothalamic receptor, Ob-Rb and endocannabinoids increase food intake by acting on cannabinoid CB_1 receptors in hypothalamus, limbic forebrain and brainstem. These anorexigenic and orexigenic mediators also modulate peripheral sweet taste sensitivity. Leptin selectively inhibits behavioral, taste nerve and taste cell responses to sweet compounds whereas endocannabinoids enhance sweet taste responses. However, potential roles of endogenous leptin and endocannabinoids in sweet taste still remain unclear. We used pharmacological antagonists for Ob-Rb (LA) and CB_1 (AM251) and examined effects of their blocking activation of endogenous leptin and endocannabinoid signaling on taste responses in lean control, leptin receptor deficient *db/db*, and diet induced obese (DIO) mice. Our results suggest that circulating leptin, but not local endocannabinoids, may be a dominant modulator for sweet taste in lean mice; however, endocannabinoids may become more effective modulators of sweet taste under conditions of deficient leptin signaling. In the gut, enteroendocrine cells express sweet taste receptor. Mouse enteroendocrine cell line STC-1 also expressed sweet taste receptor and their responses to sweet compounds were affected by leptin and endocannabinoids similar to sweet taste cells. Thus, leptin and endocannabinoids may regulate food intake and energy homeostasis via the oral-brain-gut axis.

(COI: No)

S14-2

Oscillation and synchronization of neuronal activity in the insular cortex implicated in the feeding behavior

Kang, Youngnam; Toyoda, Hiroki; Saito, Mitsuru; Sato, Hajime; Kawano, Tsutomu (*Dept Neurosci & Oral Physiol, Osaka Univ Grad Sch Dent, Osaka, Japan*)

The taste sensation arising from the taste cells in the tongue that express G-protein-coupled receptors is processed in the gustatory region of the insular cortex, while the chemosensation arising from the enterocytes in the gastrointestinal tract that express similar G-protein-coupled receptors is likely to be processed in the gastrointestinal region of the insular cortex. We found an oscillatory synchronization between the neuronal populations in the gustatory and gastrointestinal regions of the insular cortex, which may be crucial in the regulation of feeding behavior.

(COI: No)

S14-3

Cerebral cortical projections to trigeminal premotoneurons controlling jaw-movements in rats

Yoshida, Atsushi; Sato, Fumihiko; Ohara, Haruka; Fujio, Takashi; Tsutsumi, Kanako; Kato, Takafumi (*Dept. of Oral Anatomy and Neurobiology, Grad. Sch. Dent. Osaka Univ., Osaka, Japan*)

In rats, the trigeminal premotoneurons (interneurons directly projecting to the trigeminal motor nucleus [Vmo] which contains jaw-closing [JC] and jaw-opening [JO] motoneurons) were widely distributed in the intertrigeminal region (Vint), trigeminal mesencephalic nucleus (Vmes), reticular formation medial to the JO component of the Vmo (rmJO), juxtatrigeminal region (Vjuxt), trigeminal oral subnucleus (Vo), and solitary tract nucleus (Sol). The Vint and Vmes mainly contained the JC premotoneurons while the rmJO mainly contained the JO premotoneurons. The Vjuxt, Vo and Sol contained both types of the premotoneurons. The Vint received projections mainly from the lateral part of agranular cortex (Agl) which possibly corresponds to the primary somatomotor cortex (M1) while the rmJO received projections mainly from the medial part of agranular cortex (Agm); the Agm possibly corresponds to the secondary somatomotor cortex (M2) and is also considered to be a part of the prefrontal cortex involved in the autonomic and limbic function. The Vjuxt and Vo received projections mainly from the primary somatosensory cortex (S1) and Agl. The Vmes received projections mainly from the lateral part (insular cortex) and medial part of the prefrontal cortex while the Sol from the insular cortex. These findings suggest that the jaw-movements are regulated through the three types of trigeminal premotoneuron areas by the somatic sensorimotor cortex and the prefrontal cortex.

(COI: No)

S14-4

Properties of neuronal circuitry composed of supratrigeminal premotor neurons and trigeminal motoneurons

Inoue, Tomio; Nakamura, Shiro; Nakayama, Kiyomi; Mochizuki, Ayako; Yoshida, Atsushi; Kiyomoto, Masafumi (*[1]Dept Oral Physiol, Sch Dent, Showa Univ, Tokyo, Japan; [2]Dept Oral Anat, Grad Sch Dent, Osaka Univ, Osaka, Japan*)

Feeding is one of the most important survival functions for mammals. To understand neural mechanisms underlying jaw motor function during feeding, we examined properties of neuronal circuitry composed of supratrigeminal (SupV) premotor neurons and trigeminal motoneurons in rat brainstem slice preparations. SupV premotor neurons targeting the trigeminal motor nucleus (MoV) were detected on the basis of antidromic responses to electrical stimulation of the MoV using Ca^{2+} imaging and whole-cell recordings. The premotor neurons were divided into 2 groups according to their discharge patterns of the steady-state responses to 1 s current pulses: those firing higher (HF neurons) or lower (LF neurons) than 33 Hz. Intracellular labeling revealed that the axons of all HF neurons entered the MoV from its dorsomedial aspect, whereas the axons of half of the LF neurons entered the MoV from its dorsolateral aspect. Furthermore, the dendrites of a half of HF neurons penetrated into the principal sensory trigeminal nucleus, whereas the dendrites of all LF neurons were confined within the SupV. Laser photolysis of caged glutamate in the SupV induced burst excitatory postsynaptic currents especially in jaw-closing motoneurons. These results suggest that the SupV premotor neurons targeting the MoV with different firing properties have different dendritic and axonal morphologies, and these SupV neuron classes may play distinctive roles in suckling and chewing.

(COI: No)

S14-5

Effects of pharyngeal electrical stimulation on masticatory performance

Inoue, Makoto; Takeishi, Ryosuke; Hayashi, Hirokazu; Magara, Jin; Tsujimura, Takanori; Watanabe, Masahiro (*Div Dysphagia Rehab, Niigata Univ Grad Sch Med Dent Scis, Niigata, Japan*)

Purpose: The aim of this study was to examine the effects of repeated pharyngeal electrical stimulation on swallowing performance in healthy humans.
Method(s): Ten minutes pharyngeal electrical stimulation (5Hz, 1ms pulse duration) was applied in 9 healthy adults once/day for 5 days. The effects of stimulation were evaluated both on voluntary and involuntary swallowing behavior. For the effects on voluntary swallowing, the repetitive saliva swallowing test (RSST) was used, in which subjects were instructed to swallow their own saliva as quickly as possible for 30 sec and the number of swallows was counted. Changes in involuntary swallow performance was measured with the swallowing response time (SRT), where water was injected repeatedly into the pharynx at 0.1 ml/sec and the initiation latency of first swallow was measured. RSST and SRT were recorded before stimulation (baseline) and every 10 minutes up to an hour after 10-minutes stimulation.
Result(s): While SRT was not affected by pharyngeal stimulation, the number of swallows in RSST significantly increased at 60 minutes. In addition, 5-day stimulation resulted in gradual increase of number of swallows in RSST.
Conclusion: Current results suggest that repeated pharyngeal stimulation can lead to neuroplastic changes in the cortical excitability responsible for swallowing initiation.

(COI: No)

Symposium 15

Recent progress in differentiation and regeneration of vessels

(March 21, 15:30～17:00, Room E)

S15-1

Vascular morphogenesis between the brain and spinal cord in zebrafish

Kimura, Eiji (*Iwate Med. Univ., Iwate, Japan*)

Zebrafish (*Danio rerio*) is an excellent model organism to investigate developmental process of the initial vascular formation during early ontogeny because of its transparent body and exo-utero development, and it contributed to show how the cranial and truncal vasculatures were formed over the past decade. These vasculatures developed individually and conjugated at their border. However, the stepwise process bridging these systems are not uncovered enough. In this study, we demonstrated how the vascular systems of the brain and spiral cord, that is, how internal carotid arteries and vertebral arteries were integrated via basilar artery using time-lapse imaging of living transgenic zebrafish embryos, in which endothelial cells specifically expressed the EGFP. As a result, we succeeded to show the first connecting process that the primordial hindbrain channel and basilar artery extended caudally and bridged with dorsal longitudinal anastomose vessel via first intersegmental arteries. The primary connection was soon remodeled and finally internal carotid arteries were integrated with the vertebral arteries via basilar artery. The vascular cast with micro-resin revealed that the basal vasculature was conserved in adult fish. Furthermore, we confirmed the primary vascular connection was not influenced by flow dynamics, and this leads us to propose the vascular integration in this region is also controlled by genetic cues such as in the truncal region. Our morphological data of vascular formation between the brain and spinal cord will help us to understand its regulatory mechanisms.

(COI: No)

S15-2

Unveiling the cellular and molecular mechanism of vascular development by fluorescence-based bio-imaging in zebrafish

Fukuhara, Shigetomo; Mochizuki, Naoki (*Dept. Cell Biol., Natl. Cereb. Cardiovasc. Res. Inst., Osaka, Japan*)

Vascular networks develop through two distinct processes; vasculogenesis and angiogenesis. Vasculogenesis is defined as the formation of primitive vascular plexus, while angiogenesis refers to the subsequent growth and expansion of developed blood vessels. Formation of functional vasculature also requires lumen formation, arterial venous specification and pericyte coverage. However, the cellular and molecular mechanisms of vascular development in vivo remain largely unknown, because a method of addressing these questions has not been established. To overcome this problem, we have adopted fluorescence-based bio-imaging techniques using zebrafish as a model animal. To investigate the cellular and molecular mechanisms of vascular development, we have developed the transgenic zebrafish lines in which endothelial cells express various types of fluorescence-based biosensors. Those have enabled us to simultaneously visualize cellular structure, including cytoskeleton and cellular signaling, including activity of various signaling molecules and transcription factors. By performing live imaging of these transgenic lines, we successfully visualized cell-cycle progression of endothelial cells during vascular development, delineated the signaling pathways underlying the endothelial cell migration during angiogenesis and demonstrated a crucial role of beta-catenin-mediated transcription in the development of venous vessels. In this symposium, we will introduce how fluorescence-based bio-imaging technique can be exploited for vascular biology research.

(COI: No)

S15-3

Construction of biological elastic vessels by extracellular matrix nanofilm-based cell accumulation technique

Yokoyama, Utako[1]; Ishiwata, Ryo[1]; Matsusaki, Michiya[2]; Akashi, Mitsuru[2]; Ishikawa, Yoshihiro[1] ([1]*Cardiovascular Research Inst., Yokohama City Univ.;* [2]*Graduate School of Engineering, Osaka Univ.*)

Background: Construction of biological artificial vessel with high elasticity is not currently feasible within short period, and biological arterial grafts have not been available in clinical. We previously fabricated vascular smooth muscle cells (SMCs) into three-dimensional cellular multilayers (3DCMs) using a hierarchical cell manipulation technique, in which cells were coated with fibronectin-gelatin nanofilms to provide adhesive nano-scaffolds. Based on these results, we aimed to make modifications on 3DCMs to obtain biological arterial graft with high elasticity.
Methods and Results: Elastica stain and electron microscopic analysis demonstrated that 3DCMs, which consisted of seven layers of neonatal rat aortic SMCs cultured in 1% fetal bovine serum in DMEM, exhibited layered elastic fibers within 7 days of being in a static culture condition. Radioimmunoassay using [^{3}H]valine confirmed the greater amount of cross-linked elastic fibers in 3DCMs than in monolayered SMCs. However, the 3DCMs did not show measurable elasticity. We then preincubated SMCs at hyperconfluency for 5 days, followed by hierarchical cell manipulation for 7 days, and found that more differentiated SMCs provided 3DMCs with high elasticity, in which 3DCMs could be stretched more than two times in length.
Conclusion: The use of an extracellular matrix nanofilm-based cell accumulation technique provided biological elastic arterial graft within 10 days of static culture condition.

(COI: No)

S15-4

Realization of iPSC-organ bud transplantation therapy

Takebe, Takanori[1,2]; Taniguchi, Hideki[1] ([1]*Dept Reg Med, Grad Sch Med, Yokohama City Univ, Yokohama, Japan;* [2]*PRESTO, JST*)

A critical shortage of donor organs for treating end-stage organ failure highlights the urgent need for generating organs from human induced pluripotent stem cells (iPSCs). Despite many reports describing functional cell differentiation, no studies have succeeded in generating a three-dimensional vascularized organ such as liver. Towards this end, we recently established a proof-of-principle approach to grow a vascularized and functional human organ from iPSCs by transplanting in vitro-derived organ bud into immunodeficient animal (T. Takebe et al. Nature 499 (7459), 481-484, 2013). With this technology, a transplantable iPSC-derived liver bud (rudimentary liver) could be self-organized from mixed human progenitors under a specific 3-D culture platform by recapitulating early organogenetic cellular interactions. Here, I will summarize the concept and adaptability of organ bud transplantation therapy, and and share the state-of-art technology currently used by us in mass production, quality evaluation, and optimal transplantation strategies towards clinical translation. Given the unsatisfactory clinical outcomes of the cell-based therapies that are currently the main goal of stem cell therapy, this proof-of-principle could revolutionize the application of regenerative medicine in the treatment of end-stage organ failure. This technique could also elucidate aspects of human developmental biology and disease modelling, and could provide a drug-screening platform.

(COI: No)

S15-5

Generation of in vitro vascular disease models using disease-specific iPS cells

Osafune, Kenji (*CiRA.Kyoto Univ., Kyoto, Japan*)

Disease modeling research using patient-derived iPS cells has been carried out with various intractable disorders. Notably, it has already been demonstrated that the iPS disease model can provide a platform for studies aiming at both understanding pathological mechanisms and discovering new drug compounds. Autosomal dominant polycystic kidney disease (ADPKD) is the most prevalent, potentially lethal, monogenic disorder, characterized by the development of multiple renal cysts and various extra-renal manifestations. Cardiovascular complications are the main cause of death in ADPKD and intracranial aneurysms, causing subarachnoid hemorrhage, are among the most serious. The pathogenesis of vascular lesions as well as cyst formation remains largely unknown. We derived iPS cells from seven ADPKD patients, among whom four had intracranial aneurysms. These iPS cells differentiate into vascular endothelia and smooth muscle cells in vitro, which recapitulate the defective intracellular Ca2+ regulation, similar findings to those reported in vascular cells of mouse ADPKD models. Furthermore, by microarray analyses, we have identified several molecules whose expression levels are specifically altered in the iPS cell-derived vascular cells from ADPKD patients and in those from ADPKD patients with aneurysms. We are currently examining the diagnostic performance of the molecules using clinical samples. These results suggest that vascular cells differentiated from patient-derived iPS cells can be used for studying the pathogenic mechanisms of vascular diseases and for identifying possible biomarkers.

(COI: Properly Declared)

Symposium 16

Zinc signaling: An emerging regulatory system in physiology and pathogenesis

(March 21, 15:30～17:00, Room F)

S16-1

Essential role of zinc transporter-mediated zinc signaling in lymphocyte homeostasis and immunity

Fukada, Toshiyuki[1,2] (*[1]Pathology, Showa Univ Sch Dentistry, Tokyo, Japan; [2]RIKEN-IMS*)

Zinc is an essential trace element required for a variety of cellular functions and molecular events. Zinc homeostasis is controlled by zinc transporters, channels and metallothioneins, and their loss or gain of functions cause serious health problems. Recent advances of experimental approaches have unlabeled that zinc ion mediated by transporters/channels/metallothioneins acts as a signaling factor recognized as **zinc signal**, which participates in regulation of numbers of cellular phenomena, thereby in health and disease conditions. This symposium will aim to share the updated information about the significant roles of zinc signaling in cellular functions and diseases, and to discuss about the next directions and problems to be solved.
I will address that zinc transporter ZIP10-mediated zinc signaling is essential for B-cell homeostasis and related immune responses, and discuss that zinc signal selectively controls signal transduction pathways that may help us understanding the role of zinc signaling in physiology and pathogenesis.

Reference:
Proc Natl Acad Sci USA 2014; 111:11780-11785 Zinc transporter SLC39A10/ZIP10 facilitates antiapoptotic signaling during early B-cell development.
Proc Natl Acad Sci USA 2014; 111:11786-11791 Zinc transporter SLC39A10/ZIP10 controls humoral immunity by modulating B-cell receptor signal strength.
Zinc Signals in Cellular Functions and Disorders Fukada and Kambe (eds): Springer, 2014

(COI: No)

S16-2

Zinc deficiency and cutaneous immunity

Kawamura, Tatsuyoshi (*Dept Dermatol., Univ Yamanashi, Yamanashi, Japan*)

Zn deficiency can be inherited or acquired. It has many clinical manifestations, including bacterial and fungal infection of skin lesions due to impaired immune function. Despite their impaired immune function, Zn-deficient individuals develop skin inflammation (Acrodermatitis enteropathica) through an unknown mechanism. We have found that, despite diminished allergic contact dermatitis in mice fed a zinc-deficient (ZD) diet, irritant contact dermatitis (ICD) in these mice was more severe and prolonged than in zinc-adequate mice. Histological examination of ICD lesions in ZD mice revealed subcorneal vacuolization and epidermal pallor, histological features of AE. Nucleotides released from chemically-injured keratinocytes are known to serve as a causative mediator for ICD. We found that the severe ICD response in ZD mice was significantly attenuated by local injections of soluble NTPDase. In addition, the zinc-chelating reagent TPEN significantly increased ATP levels released from cultured keratinocytes treated with chemical irritants in vitro. Moreover, ZD mice exhibited significantly decreased levels of TGF-b1 in the epidermis and loss of epidermal Langerhans cells (LC), known to play a protective role against ATP-mediated inflammatory signals by hydrolyzing extracellular nucleotides. The clinical significance of these mouse data was highlighted by the observation that Zn-deficient individuals lacked epidermal LCs. Thus, our findings suggest that ATP released from injured keratinocytes, which accumulates because there are insufficient epidermal LCs to hydrolyze it, causes the skin inflammation observed in Zn-deficient individuals.

(COI: No)

S16-3

SOD1 as a molecular switch for initiating the homeostatic ER stress response under zinc deficiency

Ichijo, Hidenori; Homma, Kengo (*Lab. Cell Signaling, Grad. Sch. Pharm. Sci., Univ. of Tokyo*)

Zinc is an essential trace element, and impaired zinc homeostasis is implicated in the pathogenesis of various human diseases. However, the mechanisms cells use to respond to zinc deficiency are poorly understood. We previously reported that amyotrophic lateral sclerosis (ALS)-linked pathogenic mutants of SOD1 cause chronic endoplasmic reticulum (ER) stress through specific interactions with Derlin-1, which is a component of the ER-associated degradation machinery. Moreover, we recently demonstrated that this interaction is common to ALS-linked SOD1 mutants, and wild-type SOD1 (SOD1WT) comprises a masked Derlin-1 binding region (DBR). Here, we found that, under zinc-deficient conditions, SOD1WT adopts a mutant-like conformation that exposes the DBR and induces the homeostatic ER stress response, including the inhibition of protein synthesis and induction of a zinc transporter. We conclude that SOD1 has a function as a molecular switch that activates the ER stress response, which plays an important role in cellular homeostasis under zinc-deficient conditions.

(COI: No)

S16-4

Zinc starvation-induced autophagy in yeast

Kawamata, Tomoko; Horie, Tetsuro; Matsunami, Miou; Ohsumi, Yoshinori (*Frontier Research Center, TITECH*)

Transition metal ions such as iron, copper, zinc, and manganese are essential nutrients for every organism. Among them, zinc is abundant, essential transition metal to all forms of life. Most cellular zinc seems to be bound to intracellular ligands, such as protein. More than 5 % proteins are reported as zinc binding protein. Zinc serves as a catalytic and/or structural cofactor for many proteins. For this reason abnormal zinc homeostasis causes serious problems, including cell growth. When available zinc becomes scarce, cell must properly partitioning intracellular zinc to be prioritized. Autophagy is evolutionally conserved, cellular degradation/recycling process whereby cytoplasmic proteins and organelles are sequestered for degradation in the vacuole/lysosome. We found that autophagy has an important role to support growth under zinc starvation in yeast, which suggests that autophagy contributes to zinc ion homeostasis. Indeed, zinc depletion induces autophagy. In this symposium, we will discuss zinc economy and autophagy, and molecular mechanisms for induction of autophagy under zinc starvation in yeast.

(COI: No)

S16-5

A wide range of cellular functions of zinc transporters in the secretory pathway

Kambe, Taiho (*Grad.Sch.Biostudies.Kyoto Univ., Kyoto, Japan*)

Zinc is an essential trace element life, because it plays a pivotal role as a structural, catalytic and regulatory element in protein functions. Thus, zinc homeostasis is tightly controlled through the highly integrated processes of zinc uptake, sequestration and efflux across the cell membrane, in which zinc transporters are essential in these processes. Two solute carrier transporters, Zn transporter (ZnT) and Zrt, Irt-like protein (ZIP), primarily control zinc transports, and enable various zinc-dependent proteins functions and signalings to play physiologically important roles in numerous biological processes. A number of studies have shown that zinc mediated by ZnT and ZIP transporters has specific crucial roles in numerous cellular events. However, the molecular mechanisms have virtually remained unknown. Here, I would like to discuss this point using a model of the activation process of zinc-requiring enzymes by ZnT transporters localized to the secretory pathway. To clarify how zinc transporters mobilize zinc to a specific target protein and functions in a specific physiological role would move zinc signaling research to the next phase, and may lead to therapeutic progress.

(COI: No)

Symposium 17

Role of the auditory cortex in hearing

(March 21, 15:30～17:00 Room G)

S17-1

Anatomical study on neural circuits of the mouse insular auditory field

Takemoto, Makoto; Hasegawa, Kayoko; Song, Wenjie (*Dept Sens Cogn Physiol, Grad Sch Med Sci, Kumamoto Univ, Kumamoto, Japan*)

The auditory cortex is divided into several subfields based on their response properties. The role of each subfield is a most intriguing issue in the research on the auditory cortex, and neuronal connectivities of these subfields can help gain an insight into their characteristic functions. We have recently identified an insular auditory field (IAF) in mice. To reveal the neural circuits of the IAF, we performed retrograde and anterograde neuron tracing by using fluorophore-conjugated cholera toxin subunit B and adeno-associated virus vectors encoding fluorescent protein genes, after area identification by optical imaging. We show that the IAF receives input from a ventromedial portion of the ventral division of the medial geniculate body, a core auditory thalamic nucleus. Moreover, distinct subsets of IAF neurons send their axons to the motor cortex or dysgranular insular cortex that contains medulla-projecting neurons. These results suggest that the IAF activated by auditory input could modify motor and autonomic functions.

(COI: No)

S17-2

Re-definition of the primary auditory cortex by separating a newly identified region and their functional specialization in mice

Tsukano, Hiroaki; Shibuki, Katsuei (*Dept of Neurophysiol, Brain Res Inst, Niigata Univ, Niigata, Japan*)

The auditory cortex is composed of several regions in mice. However, the regional function is yet to be identified clearly because delineation of regions including the primary auditory cortex (AI) is still under debate. We investigated the mouse auditory cortex using flavoprotein fluorescence imaging. AI was divided into two areas, dorsoventrally, in response to high frequency tones. We performed immunostaining using SMI-32 and found the dorsal AI high frequency area had totally different cytoarchitectural patterns from those of the rest of the AI, i.e., the low frequency area and ventral high frequency area. This data suggests that the AI dorsal high frequency area is a distinct region to be separated from the AI. We named this new region the dorsomedial field (DM). The responses of the DM to ultrasonic courtship songs presented by males were significantly greater in females than in males. In contrast, there was no difference between sexes in responses to artificial pure tones. These data suggest that courtship songs by male mice might be processed in DM. On the other hand, AI is likely to process harmonic sounds. Harmonic sounds of simultaneously presented 20 kHz and 25 kHz, which produced missing f0 perception at 5 kHz, activated the 5 kHz area of AI, while inharmonic sounds of simultaneously presented 19 kHz and 26 kHz did not. Furthermore, in mice reared in the presence of 5+19+26 kHz, the 5 kHz area in AI responded to inharmonic sounds of 19+26 kHz. These results indicate importance of experience for producing f0 responses.

(COI: No)

S17-3

Sound coding in auditory cortex; studies from single unit activities in the primary auditory cortex (A1) of awake animals

Chimoto, Sohei (*Dept Physiol, Interdisciplinary Grad Sch of Med and Eng, Univ of Yamanashi, Chuo, Yamanashi, Japan*)

To reveal the mechanism of sound coding in auditory cortex, many studies have investigated neural responses to various sound stimuli by using electrophysiological techniques of single unit recordings in awake cats. A1 neurons showed diversity of the response time-courses from phasic to sustained patterns to pure tone stimuli. The sustained response cells have different spectral edge sensitivities. The edge-sensitive cells had tuning to the high-edge or low-edge frequencies of sound stimuli, while the edge-insensitive cells were driven by any stimuli with energy on the cell's frequency response field (FRF) or only very narrowband stimuli with energy confined to FRF. They have different sensitivities to the fundamental frequency (F0) of harmonic complex tones. The F0-sensitive cells discriminated between harmonics and noise, while the F0-insentive cells did not. All aspects of the sustained responses were consequences of the spectral filtering properties. The phasic response cells showed paired onset and offset responses. Their frequency filtering property was dynamic, changing between sound onsets and offsets. Each response was precise and salient for effectively encoding sound onsets and offsets. A1 neurons showed various response patterns during amplitude modulation sounds, frequency modulation tones, and natural sounds. The presence of phasic and sustained response cells explained the diversity of response time courses for the various sounds. The same cell responds to pure tone and other kinds of sound in the specific response pattern.

(COI: No)

S17-4

Steady-state neuronal activity pattern in response to long-lasting continuous tone in the auditory cortex of rat

Takahashi, Hirokazu (*Research Center for Advanced Science and Technology, The University of Tokyo, Japan*)

Acoustic stimuli elicit distinct onset responses in the auditory cortex, which were followed by obscure long-latency responses. The onset responses exhibit clear tonotopic map and learning induced plasticity, which have been fully investigated to date. On the other hand, post-onset, long-latency responses have been poorly characterized because of less reproducibility across trials. Our conscious experience that lasts beyond the time course of onset responses implies that the long-latency responses play an important role in perception. In the present study, we investigate whether and how post-onset, steady-state activities in response to long-lasting continuous tones encode sound information and exhibit learning-induced plasticity. A microelectrode array with a gird of 10 × 10 recording sites was inserted into the 4th layer of the auditory cortex of rats to record local field potentials (LFPs) within the steady-state activity. For all possible pairs of recording sites, band-specific phase locking values (PLVs) of measured LFPs were calculated. We demonstrate that the PLV pattern represented rich information about sound such as frequency, consonance and tonality of chords. Furthermore, appetitive and aversive classical conditioning modified these patterns differently. These results support our hypothesis that long-latency, steady-state responses play important roles in encoding auditory information.

(COI: No)

Symposium 18

Mechanism of host defence and homeostatic maintenance by phagocytes

(March 21, 15:30～17:00, Room H)

S18-1

Multistep regulation of ROS production by voltage-gated proton channel Hv1/VSOP in neutrophils

Okochi, Yoshifumi[1]; Aratani, Yasuaki[2]; Adissu, Hibret A[3]; Miyawaki, Nana[1]; Sasaki, Mari[1]; Suzuki, Kazuo[4]; Okamura, Yasushi[1] (*[1]Integrative Physiol, Grad Sch Med, Osaka Univ, Osaka, Japan; [2]Grad School of Nanobioscience, Yokohama City Univ, Yokohama, Japan; [3]Physiol & Experimental Med, Hospital for Sick Children, Toronto, Canada; [4]Asia International Institute of Infectious Disease Control, Teikyo University, Tokyo, Japan*)

Reactive oxygen species (ROS) is a strong weapon for pathogen killing in phagocytes, whereas ROS is known to damage host itself. The ROS in neutrophils is generated by NADPH oxidase and myeloperoxidase, and it production is strictly regulated by various factors and ways such as pH, membrane potential and granule exocytosis. We discovered the molecule of voltage-gated proton channel Hv1/VSOP and have been analyzing the function of Hv1/VSOP in cellular and animal levels using Hv1/VSOP knockout mouse. We and collaborators have reported that Hv1/VSOP helps O_2^- and H_2O_2 productions through the regulation of intracellular pH and membrane potential in neutrophils, where these factors are known to affect the activity of NADPH oxidase that produce O_2^-. Recently, we found that Hv1/VSOP regulates ROS production in another way: Hv1/VSOP negatively regulates HOCl production, which is made from H_2O_2 by myeloperoxidase, by inhibiting exocytosis of myeloperoxidase-containing granules (azurophilic granules). These results indicate that Hv1/VSOP controls each ROS production by multiple means in neutrophils. Hv1/VSOP may be necessary for balancing competing goals for pathogen killing and protection of host in ROS production.

(COI: No)

S18-2

Optogenetic analysis of spatiotemporal regulation of macropinocytosis and phagocytosis through Rac1 switching in macrophages

Araki, Nobukazu (*Sch.Med.Kagawa Univ., Miki, Japan*)

In phagocytes such as macrophages, macropinocytosis of fluid-phase and phagocytosis of particulate materials play pivotal roles in the immune system. Mechanisms of both macropinosome and IgG-mediated phagosome formation share many common features including actin cytoskeleton dependence and its regulatory components, as well as a large vacuole size (larger than 0.5 μm), although there are some differences in detail. Recently, we have investigated the molecular mechanisms underlying macropinosome and phagosome formation which are spatiotemporally coordinated by dynamic actin organization. Rac1, a member of the Rho family GTPases, acts as a molecular switch which regulates actin polymerization and remodeling. Optogenetics of photoactivatable Rac1 (mCherry-LOV-Rac1Q61L) enables us to manipulate Rac1 activity both in space and time using blue light irradiation under a microscope. Using this technique, we could induce cell surface ruffling and macropinocytic cup formation by photoactivation of Rac1 in RAW264 macrophages. Although the number of macropinocytic cups increased with prolonged time of Rac1 photoactivation, the cup closure into macropinosomes was restrained. When the blue light irradiation was turned off, ruffling immediately receded. Thereafter, macropinosomes were formed by closing cups. In the case of Fc γ R-mediated phagocytosis, activation of Rac1 facilitated pseudopod extension around IgG-opsonized particles. However, subsequent deactivation of Rac1 was also required for shaping phagocytic cups tightly grasping IgG-opsonized particles.

(COI: No)

S18-3

Targeting and assembling mechanisms of NADPH oxidases at phagosomal and apical membranes

Ueyama, Takehiko; Saito, Naoaki (*Biosignal Res. Ctr., Kobe Univ., Kobe, Japan*)

Among the seven known mammalian Nox family NADPH oxidases (Nox1-Nox5, Duox1, Duox2), several are known to serve essential roles based on the effects of disease-related mutations. Defects in the phagocytic oxidase (Nox2) have been known resulting in chronic granulomatous disease (CGD), which is characterized by susceptibility to infection. A Nox3-based oxidase is indispensable for development of otoconia. Mutations in Duox2 have been reported to cause congenital hypothyroidism due to deficiency of thyroid hormone biosynthesis. We have explored targeting and assembly of Nox2, Nox3, and Duoxes.In the phagocytic oxidase, cytosolic regulators (p47phox, p67phox, p40phox, Rac, PKC) translocate and associate with the membrane-spanning flavocytochrome b558, leading to superoxide production. The ternary complex (p47phox-p67phox-p40phox), Rac, and PKC translocate to phagosomes with independent mechanism. Although p67phox is not targeted to phagosomes by itself, p47phox functions as an adaptor for the ternary complex in early stages of phagocytosis, while p40phox functions in later stages. Duoxes are functional only in combination with Duox activators (DuoxAs), and work on the apical surfaces of epithelial cells. Besides, NADPH oxidases produce the primary product superoxide by directly transferring an electron to O2. Duoxes produce superoxide as an intermediate product, but the final product generated by Duoxes is H2O2.In this symposium, I will demonstrate targeting mechanisms of Nox2 to phagosomes and Duoxes to apical membranes, and also conversion mechanism of superoxide to H2O2 by Duoxes.

(COI: No)

S18-4

Splenic dendritic cells phagocytose donor T-cells and induce anti-donor MHC antibody forming cell response

Matsuno, Kenjiro; Ueta, Hisashi; Kitazawa, Yusuke; Sawanobori, Yasushi (*Dokkyo Med.Univ., Tochigi, Japan*)

Aim: Donor-specific blood transfusion (DST) is one of the tolerance-inducing protocols used in clinical transplantation, where a donor blood transfusion can readily induce alloantibody production in recipients. In this study, we examined a mechanism for this antibody response in rats and mice.

Result: DST-treated recipients produced donor class I MHC antigen (MHCI)-specific alloantibodies. Among the donor blood components, T-cells were the most efficient immunogens. One day after the DST treatment, donor T-cells migrated to the splenic periarterial lymphocyte sheath (PALS), where donor MHCI+ fragments were found within the resident DCs. Resident DCs formed clusters with BrdU+ cells, where the recipient CD4+ T-cell proliferative response begun. From day 3, antibody-forming cell response begun followed by the germinal center reaction at day 5. Inhibition of T-cell entry from the red pulp into the PALS strongly suppressed the DST-antibody response. In mice, two-photon intravital microscopy elucidated that the resident DCs readily bound to donor allogeneic T-cells and then ingested them.

Conclusion: Donor T-cells can induce alloresponse most efficiently, because the trafficking pattern of recirculating T-cells is designed to freely enter the PALS and cluster with the resident DCs, thus having a chance to provide alloantigen to DCs. Recirculating T-cells pulsed with antigens might be applicable as vaccine vectors, targeted to the resident DCs for the prophylactic antibody production. (Collaborators: Tomoya Katakai and Takamasa Ueno)

(COI: No)

Symposium 19

Physiological functions of membrane transporters that regulate signals for tooth morphogenesis and differentiation

(March 21, 15:30～17:00, Room I)

S19-1

Analysis of tooth development and bone remodeling using a3 isoform of V-H$^+$ATPase -GFP and -deficient mice

Harada, Hidemitsu[1]; Fujiwara, Naoki[1]; Otsu, Keishi[1]; Sahara, Yoshinori[2]; Horie, Sawa[2]; Nakanishi, Mayumi[3]; Matsumoto, Naomi[3]; Ohshima, Hayato[4]
(*[1]Dpt. Anat., Iwate Med. Univ., Iwate; [2]Dpt. Physiol., Iwate Med. Univ., Iwate; [3]Fac Pharm. Sci., Iwate Med. Univ., Iwate; [4]Niigata Univ. Grad. Sch. MDS, Niigata*)

Vacuolar proton-ATPase (V-H$^+$ATPase) is a multi-subunit enzyme that regulates proton transport and creates the acidic microenvironment. Recently, it has been reported that the strong expression of the a3 isoform is closely associated with the exocytosis of some cells. To examine the localization and function of a3 isoform in the bone and tooth development, we used a3 isoform-GFP and -deficient mice. The strong expression of GFP was detected specifically at osteoclasts, but not dental epithelial cells. The size of body and head of a3 isoform-deficient mice was small, and the tooth eruption was inhibited or delayed, and the root was often morphologically short and anomaly. Though the tooth germs grew normally until bell stage in the development, ameloblasts structurally was unaffected and the mineral content of enamel was similar to that of wild type mice. Interestingly, the bone mineral content of the mutant was lower than that of wild type unexpectedly. Furthermore, to examine the character of the dental epithelial cells in detail, we produced a dental epithelial cell line from the mutant mice and compared the cell line with wild type cell line about organelle acidification. The results showed that there is no difference between these cells. Taken together, it is considered that the decline of bone metabolism resulted in tooth anomaly.

(COI: No)

S19-2

Role of glucose metabolism in amelogenesis

Ida-Yonemochi , Hiroko (*Niigata Univ. Grad. Sch. of Med. and Dent. Sci., Niigata, Japan*)

In organogenesis, cells exhibit various behaviors, such as proliferation, changes in cell shape, matrix production and secretion. It is generally believed that many cells utilize glucose as the basis of energy, and the glucose metabolic pathway is a critical event in determining cell behavior in organogenesis. Blood glucose is transported into cells by glucose transporters (GLUTs), and GLUTs are expressed in tissue- and cell-specific functional manners depending on various glucose requirements. Recently, we demonstrated that the expression of GLUT1/2 in the dental cells is precisely and spatiotemporally controlled depending on cell differentiation. In an in vitro organ culture experiment with an inhibitor of GLUTs1/2, the bud-stage tooth germs showed the developmental arrest of the explants. And the inhibition of GLUTs1/2 in cap-to-bell-stage tooth germs reduced tooth size. These findings suggest that the glucose uptake mediated by GLUT1/2 plays a crucial role in the early tooth morphogenesis and tooth size determination. Next, we examined the glucose metabolism in amelogenesis. We found that the timing of glycogen synthesis, accumulation and degradation is also tightly associated with the process of ameloblast differentiation. In vitro organ culture experiment, the inhibition of glycogen synthesis/degradation disturbed ameloblast differentiation and enamel matrix formation, and the activation of Akt signaling by IGF-1 consequent glycogen accumulation led to an increase in enamel matrix formation. Thus, the glycogen shunt governed by IGF-1-Akt signaling is an essential system for ameloblast differentiation.

(COI: No)

S19-3

Regulation of BMP-2 expression by extracellular-calcium ions/-phosphate ions in dental pulp cells

Nemoto, Eiji (*Grad.Sch.Dent.Tohoku Univ., Sendai, Japan*)

Dental pulp cells, which have been shown to share phenotypical features with osteoblasts, are capable of differentiating into odontoblast-like cells and generating a dentin-like mineral structure. Elevated extracellular calcium ($Ca^{2+}{}_o$) and extracellular phosphate (Pi) are known to play key roles in promoting osteoblastic differentiation by altering gene expression and cellular function; however, the roles of $Ca^{2+}{}_o$ and/or Pi signaling in odontogenesis remain unclear. We found that elevated $Ca^{2+}{}_o$ as well as Pi increase the gene expression of bone morphogenetic protein (BMP)-2, a crucial regulator of mineralization, in human dental pulp cells. The $Ca^{2+}{}_o$-mediated *BMP-2* increase was markedly inhibited by pretreatment with an extracellular signal-regulated kinase (ERK) inhibitor, PD98059, and partially inhibited by the L-type Ca^{2+} channels inhibitor, nifedipine. However, pretreatment with nifedipine had no effect on ERK1/2 phosphorylation triggered by Ca^{2+}, suggesting that the Ca^{2+} influx from Ca^{2+} channels may operate independently of ERK signaling. On the other hand, Pi-mediated *BMP-2* expression requires activation of cAMP/protein kinase A, which is indispensable but not sufficient for the *BMP-2* increase. Moreover, the *BMP-2* increase requires activation of ERK1/2 pathway, which may operate independently of cAMP-dependent signaling. Importantly, it may be possible to use this knowledge as a means to deliver $Ca^{2+}{}_o$ and Pi to local sites to regenerate mineralized tissues associated with the oral cavity.

(COI: No)

S19-4

Intercellular odontoblast-neuron signal communication via ATP

Sato, Masaki (*Dept Physiol, Tokyo Dent Col, Japan*)

Various stimuli to the dentin surface induce dentinal pain. There is, however, no clarity regarding the precise mechanisms of dentinal pain generation as well as the role of odontoblasts in the underlying sensory transduction pathway. In order to determine if odontoblasts act as sensory receptors in this pathway, we mechanically stimulated odontoblasts and investigated transient receptor potential (TRP) channel activation in these cells and the subsequent intercellular signaling between odontoblasts and neurons. Direct mechanical stimulation of single odontoblasts increased intracellular free calcium concentration ($[Ca^{2+}]_i$) via TRP channel activations. In a co-culture of odontoblasts and trigeminal ganglion (TG) neurons, direct mechanical stimulation of single odontoblasts increased $[Ca^{2+}]_i$ not only in the stimulated odontoblasts, but also in neighboring odontoblasts and TG neurons. The $[Ca^{2+}]_i$ increase in neighboring odontoblasts and TG neurons, but not that in stimulated odontoblasts, was inhibited by a pannexin-1 inhibitor. A $P2X_3$ receptor antagonist suppressed the $[Ca^{2+}]_i$ increase in neighboring TG neurons, but not in stimulated and neighboring odontoblasts. These results show that TRP channel activation in mechanically stimulated odontoblasts results in ATP release via pannexin-1 and the ATP transmits a signal to the $P2X_3$ receptors on TG neurons. These results also enhance understandings sensory of transduction sequence for dentinal pain, termed as "odontoblast hydrodynamic receptor theory".

(COI: No)

Symposium 20

A better understanding of liver metabolism by multifaceted approaches

(March 21, 15:30～17:00, Room J)

S20-1

Metabolite sensing and regulation of glucose metabolism in liver

Miki, Takashi[1]; Lee, Eunyoung[1]; Minokoshi, Yasuhiko[2] ([1]*Dept Med Physiol, Grad Sch Med, Chiba Univ, Chiba, Japan;* [2]*Dept Dev Physiol, NIPS, Okazaki, Japan*)

Liver plays a central role in the regulation of glucose metabolism. We found that the insulin receptor mutant mice (mIR) exhibit marked hyperglycemia under high fat diet (HFD). In mIR under HFD (mIR/HFD), lipolysis in white adipose tissues (WAT) and mRNA expression of glucose-6-phosphatase (G6pc) in liver were both increased on refeeding. Glycerol is the end product of lipolysis and insulin inhibits lipolysis by reducing phosphorylation of hormone sensitive lipase (HSL). As expected, phospho-HSL levels were significantly increased in WAT of mIR/HFD. In addition, transplantation of wild-type WAT into mIR/HFD markedly ameliorated the hyperglycemia, suggesting the pathophysiological relevance of unsuppressed lipolysis on development of hyperglycemia in mIR/HFD. Nevertheless, adipocyte specific insulin receptor knockout mice did not exhibit glucose intolerance. This prompted us to hypothesize that insulin dysfunction in liver is also involved in the pathogenesis. We found that glycerol administration induces mRNA expression of G6pc in to wild-type mice, suggesting that hepatocyte senses glycerol or its derivatives to regulate glucose metabolism. In addition, defective insulin action in liver is suggested to participate in the increased gluconeogenesis from glycerol. In this symposium, the mechanism of nutrient sensing in liver and its relevance in glucose homeostasis will be discussed.

(COI: No)

S20-2

Vitamin A-storing lipid droplets in hepatic stellate cells

Yoshikawa, Kiwamu[1]; Mezaki, Yoshihiro[1]; Hebiguchi, Taku[2]; Miura, Mitsutaka[1]; Imai, Katsuyuki[1]; Yamaguchi, Noriko[3] ([1]*Dep. Cell Biol. and Morp., Grad. Sch. of Med., Akita Univ.;* [2]*Dep. Ped. Sur., Grad. Sch. of Med., Akita Univ.;* [3]*Dep. Bas. Nurs., Grad. Sch. of Med., Akita Univ.*)

Hepatic stellate cells (HSCs) lie in the perisinusoidal space in the liver and store about 50-80% of total body vitamin A as retinyl esters in their cytoplasmic lipid droplets (LDs) in mammals. Therefore the vitamin A-storing LDs are considered specialized organelle for vitamin A storage. Under pathological conditions such as liver fibrosis, HSCs proliferate rapidly and produce large amounts of extracellular matrix components and lose their stored vitamin A (activation of HSCs). We investigated the involvement of perilipin 2 (ADRP) and perilipin 3 (TIP47) in the formation of vitamin A-storing LDs in cultured rat HSCs and observed very characteristic behaviors of the proteins. Perilipin 2 always localized on almost all vitamin A-storing LDs in any conditions examined; resting, growing (supplement of retinol and/or oleate), and diminishing conditions in both quiescent and activated HSCs. Perilipin 3 rarely localized on vitamin A-storing LDs in quiescent HSCs. However in activated HSCs it localized on newly induced vitamin A-storing LDs from small to very large ones as long as the constituents of LDs were supplied. In consequence, perilipin 2 and 3 co-localized on the same LDs for a long period irrespective of their size. Vitamin A-storing LDs positive for perilipin 3 were decreased after supplement of LD constituents was stopped. The manner of involvement of the two proteins for LD formation in HSCs will be discussed.

(COI: No)

S20-3

UBXD8 function in liver

Suzuki, Michitaka; Imai, Norihiro; Ohsaki, Yuki; Cheng, Jinglei; Fujimoto, Toyoshi (*Grad. Sch. Med. Nagoya Univ., Nagoya, Japan*)

Apolipoprotein B (ApoB) is the principal protein of VLDL, and lipidated cotranslationally by the microsomal tryglyceride transfer protein (MTP) activity in ER in hepatocyte. Previously we found that ApoB after lipidation accumulates in the ER fused to LDs (named ApoB-crescent structures) upon inhibition of proteasomes or autophagy and is partially ubiquitinated. The result suggested that ApoB-crescent structures function as a platform of lipidated ApoB degradation.

To elucidate the molecular mechanism of lipidated ApoB degradation, we searched for proteins that are engaged in the process and found UBXD8, p97 and Derlin-1. Knockdown of Derlin-1 increased ApoB in the ER lumen of ApoB-crescent structures. In contrast, knockdown of UBXD8 reduced p97 recruitment to LDs, and caused ApoB accumulation not only in the ER lumen but also on the LD surface facing the cytoplasm. Furthermore, UBXD8 bound ubiquitinated ApoB. The results inferred that UBXD8 together with p97 and Derlin-1 facilitates retrotranslocation and proteasomal degradation of lipidated ApoB in the vicinity of LDs.

To evaluate the physiological function of UBXD8 in liver in vivo, we generated hepatocyte-specific UBXD8 knockout (UBXD8-LKO) mice. After twenty-six weeks of high-fat diet feeding, VLDL secretion and the serum TG level were reduced in the UBXD8-LKO mice compared to the wild-type mice. Furthermore, the UBXD8-LKO mice showed steatosis both in the periportal area and the perivenular area, whereas the control mice showed steatosis only in the perivenular area. These data indicated that UBXD8 plays an important role in regulating ApoB secretion from hepatocytes.

(COI: No)

S20-4

Mechanism for biliary phospholipid efflux mediated by ABCB4 on canalicular membranes

Morita, Shin-ya (*Shiga University of Medical Science, Otsu City, Shiga, Japan*)

Bile salts have potent detergent properties and damage hepatocytes by affecting the integrity of cellular membranes, which are associated with hepatocellular necrosis. On the canalicular membranes of hepatocytes, ABCB4 plays an essential role in the secretion of phospholipids into bile. The biliary phospholipids are associated with bile salts and cholesterol in mixed micelles, thereby reducing the cytotoxicity of bile salts and preventing cholesterol crystallization. Mutations in the ABCB4 gene result in cholestasis, cholelithiasis, and cholangiocarcinoma. To elucidate the mechanism for ABCB4-mediated phospholipid efflux, we established HEK293 cells stably expressing ABCB4. Surprisingly, the phospholipid efflux from ABCB4-expressing cells was remarkably enhanced by taurocholate. ABCB4 mutants in ATP-binding domains did not mediate the phospholipid efflux. Mass spectrometry revealed that ABCB4-expressing cells preferentially secreted phosphatidylcholine (PC) rather than sphingomyelin (SM). In addition, by using enzyme-based fluorometric methods, we demonstrated that taurocholate stimulated the ABCB4-mediated efflux of PC, phosphatidylethanolamine (PE) and SM, while the efflux of PE and SM was much less than that of PC. ABCB4 was predominantly localized to nonraft membranes, and the ABCB4-mediated phospholipid efflux was completely abolished by BODIPY-verapamil, which hardly partitioned into raft membranes. In conclusion, ABCB4 localized in nonrafts, but not in rafts, mediates the efflux of phospholipids, preferentially PC, only in the presence of bile salts.

(COI: No)

Symposium 21

Space Medicine II: Complications of "Zero-Gravity" and their countermeasures

(March 21, 17:00～18:30, Room B)

S21-1

Medical challenges that need to be solved in super-long-duration stays in space facing Human Space Exploration

Furukawa, Satoshi; Ohshima, Hiroshi; Ogata, Katsuhiko; Miki, Takeo; Suzuki, Go; Abe, Takashi; Ohira, Takashi (*JAXA, Tsukuba, Japan*)

At the International Space Exploration Forum in January, 2014, there was a minister-level policy dialogue on international cooperation in space exploration. Long stay in space in human space exploration will cause physiological changes to human body. Below are examples. They are challenging and countermeasures are needed.1.MusculoskeletalOn International Space Station (ISS) muscle atrophy and bone loss induced by zero-G is almost controlled by good exercise devices. As for space exploration, limited room may require more compact ones. Plus, a countermeasure drug /food would be nice.2.NeurovestibularIt takes several months to get to Mars. On the way being exposed to zero-G, astronauts' vestibular system is adapted to zero-G. Arriving at Mars, astronauts experience "readaptation to gravity sickness". They need to work without the support of rescue team. Certain measures should be implemented.3.Immune SystemIn space, reduced number and proportion of lymphocytes and their cytokine production, decreased neutrophil and monocyte functions, decreased NK cell cytotoxicity were noted. JAXA is starting research on enterobacterial flora and immunity.4.RadiationAstronauts are subject to large amounts of space radiation due to lack of protection by the atmosphere or magnetic field around the Earth. Physical radiation shielding or modification of exposure by a drug/food would be great.5.VisualMore than 30% of ISS astronauts experience chronic visual acuity changes with papilledema and/or choroidal folds. The cause is not well known.

(COI: No)

S21-2

Our Space Biology Experiments in "Kibo (JEM)" of the International Space Station to Conquer Spaceflight-Associated Diseases

Nikawa, Takeshi[1]; Sokabe, Masahiro[2] ([1]*Dept Nutr Physiol, Inst Med Nutr, Tokushima Univ.;* [2]*Dept Biol Physics, Faculty Med, Nagoya Univ.*)

Skeletal muscles are vulnerable to rapid and marked atrophy under microgravity conditions. Unfortunately, despite the fact that almost of all astronauts are afflicted by debilitating atrophy, no treatment besides training exists to halt or reverse its progression, besides training. The muscle atrophy caused by microgravity is characterized by both decreased responsiveness to myogenic growth factors (e.g., IGF-1 and insulin) and increased proteolysis. Previously we showed that simulated microgravity conditions, such as tail-suspension and three dimensional (3D)-clinorotation, resulted in skeletal muscle atrophy through the induction and activation of the ubiquitin ligase Cbl-b. Upon induction, Cbl-b interacted with and degraded the IGF-1 signaling intermediate IRS-1. In turn, the loss of IRS-1 activated the FOXO3-dependent induction of atrogin-1/MAFbx, a dominant mediator of proteolysis in atrophic muscle. Furthermore, Cbl-b-deficient mice were resistant to tail-suspension-induced muscle atrophy and the loss of muscle function. Thus, the Cbl-b-dependent destruction of IRS-1 is a potent dual mediator of both increased protein degradation and reduced protein synthesis observed in microgravity conditions. In our space experiments, named "Myo Lab" and "Cell Mechanosensing", we aim to elucidate this hypothesis on "Microgravity-mediated Cbl-b expression" and Cbl-b-mediated skeletal muscle atrophy to develop a new therapeutic strategy (drug discovery in space) for muscle atrophy.

(COI: Properly Declared)

S21-3

Study and development of the countermeasure device on the disuse atrophy of the musculoskeletal system of the astronauts staying in the space for a long term -Participating in International Anouncement utilizing International Space Station-

Shiba, Naoto[1]; Matsuse, Hiroo[1]; Takano, Yoshio[2]; Omoto, Masayuki[1]; Hashida, Ryuuki[1]; Yamada, Shin[3]; Ohshima, Hiroshi[3] ([1]*Dept Orthop Surg, Kurume University School of Medicine;* [2]*Dept of Physical Therapy, Fukuoka Branch of Internatinal University of Health and Welfare;* [3]*Japan Aerospace eXploration Agency*)

The hybrid training system: HTS was developed as a countermeasure for the musculoskeletal atrophy common to astronauts and uses a contraction produced in a muscle by electrical stimulation of antagonist to resist the volitional contraction of the agonist. HTS, which utilizes electrically stimulated antagonist force as resistance to joint motion instead of gravity, would become a useful training method in weightlessness. In this experiment, HTS will be used for one of upper limbs of an astronaut (the non-dominant arm) for four weeks, and his/her muscular strength and bulk will be compared to those of his/her non-HTS arm (the dominant arm) to examine its orbital operation capability utility, as well as the preventive effect of HTS for musculoskeletal atrophy. The initial flight data together with the wealth of ground data obtained so far will be brought to the future planning of this project after the first flight.HTS will become a useful back-up for the large standard training devices in the ISS, or a useful training device in small space ships (CTV) for the exploration of the Moon and Mars.

(COI: No)

S21-4

Effects of artificial gravity by short arm centrifuge of 1.4 m with exercise as the countermeasures for spaceflight deconditioning

Iwase, Satoshi; Nishimura, Naoki (*Dept Physiol Aichi Med Univ*)

Crew members often suffer from space flight deconditioning including neurovestibular disorientation, cardiovascular deconditioning, myatrophy, and bone mineral loss. Several countermeasures have been introduced, but no single measure has been proved to be effective as the countermeasure. We have constructed the short arm centrifuge with ergometer with the radius of 1.4 m because the available space in international space station was reported to the inside of the cylinder with the diameter of 2.8 m. Subjects were required to lay down with supine position. Their legs were raised up to 70 cm high, and there, cycling pedals, which was fixed at the level of leg rotation, were stepped during centrifuge. G-level of 1.4 G with ergometric exercise of 60 W was loaded in the countermeasure group while control group were requested to lie down without exercise. Several measurements were applied to assess neurovestibular, cardiovascular, musculoskeletal, and bone metabolism, and bedrest studies were carried out comparing the countermeasure and control groups. As the results, centrifuge-induced artificial gravity with exercise has been provided significant difference from the control. We concluded artificial gravity is effective in mitigating spaceflight deconditioning in humans.

(COI: No)

S21-5

Long term stay in microgravity-induced suppression of vestibular function and its countermeasure

Morita, Hironobu; Abe, Chikara; Tanaka, Kunihiko (*Dept Physiol, Gifu Univ Grad Sch Med, Gifu Japan*)

Gravity, not only changes in amount but also changes in direction, is a major and the most common disturbance for our daily life. To cope with this, the vestibular system plays an important role in controlling body balance and arterial pressure upon gravitational stress. However, the vestibular system is known to be highly plastic, thus it is possible that the vestibular function might be altered, if subjects were in a different gravitational environment. This is true, since vestibular-mediated motor coordination and vestibulo-cardiovascular reflex were suppressed in rats raised in hypergravity for 2 weeks. Furthermore, vestibulo-cardiovascular reflex was also suppressed in astronauts who stayed in the International Space Station for 4-6 months. Thus, both hypergravity and microgravity elicit the suppression of the vestibular function, which might be due to use-dependent plasticity of the vestibular system, since phasic input to the vestibular system was reduced not only in microgravity but also hypergravity (less than 20% of 1 g environment). If this is the case, the suppressed vestibular function could be ameliorated by an artificial vestibular stimulation. Indeed, hypergravity-induced suppression of vestibular-mediated motor coordination and vestibulo-cardiovascular reflex were ameliorated by galvanic vestibular stimulation (GVS). Because posture imbalance and orthostatic intolerance are major complications of spaceflight, it is urgent to propose an effective countermeasure against the suppressed vestibular function, and GVS can be a candidate for this.

(COI: No)

Symposium 22

The effect of perinatal stress on brain function

(March 21, 17:00～18:30, Room C)

S22-1

Analyses of the effects of embryonic ischemia on brain development

Kubo, Ken-ichiro[1]; Deguchi, Kimiko[1]; Nagai, Taku[2]; Aramaki, Michihiko[1]; Yamada, Kiyofumi[2]; Inoe, Ken[3]; Nakajima, Kazunori[1] (*[1]Dept. Anatomy, Keio Univ. Sch. Med., Tokyo, Japan; [2]Dept. Neuropsychopharmacol. & Hospital Pharmacy, Nagoya Univ. Sch. Med., Nagoya, Japan; [3]Department of MR and BDR, NIN, NCNP, Tokyo, Japan*)

Environmental factors cause various neuropsychiatric diseases, such as schizophrenia, autism, and mood disorders, but their influence on brain development is largely unknown. To analyze the pathological mechanisms of environmental factors during brain development in detail, we produced ischemic brain damage in developing mouse embryos by occluding maternal uterine arteries. By combining *in utero* electroporation technique for tracking and visualization of neurons, we identified delayed neuronal migration and disruption of the final "inside-out" pattern of cortical neuronal alignment. We also performed behavioral analyses at postnatal stages to assess the neuropsychiatric functions of mice that had experienced ischemia during embryonic period. Importantly, cognitive impairments were associated with the mice that had experienced ischemia during development. Since cognitive deficits are known to underlie various neuropsychiatric diseases, ischemic insults in the developing cerebral hemispheres are thought to affect neurodevelopment, resulting in subsequent cognitive impairment that lead to increased susceptibility to neuropsychiatric diseases.

(COI: No)

S22-2

Effects of early life adverse experiences on the brain: Implications from maternal separation models in rodents

Nishi, Mayumi; Horii, Noriko; Sasagawa, Takayo (*Nara Med. Univ., Kashihara, Japan*)

During postnatal development, adverse early life experiences affect the formation of neuronal networks and exert long-lasting effects on neural function. Many studies have shown that daily repeated maternal separation (MS), an animal model of early life stress, can regulate the hypothalamic-pituitary-adrenal axis (HPA axis) and affect subsequent brain function and behavior during entire life including puberty and adulthood. However, the molecular basis of the long-lasting effects of early life stress on brain function has not been fully elucidated. In this symposium, we will present various cases of MS in rodents and illustrate the alterations in HPA axis activity by focusing on corticosterone (CORT). We then show a characterization of the brain regions affected by various patterns of MS, including repeated MS and single time MS at various stages before weaning, by investigating a neuronal activity marker, c-Fos. These CORT and c-Fos studies suggest that repeated early life stress may affect neuronal function in region- and temporal-specific manners, indicating a critical period for habituation to early life stress. Next, we introduce how early life stress can impact behavior, namely by inducing depression, anxiety or eating disorders, and alterations in gene expression in adult mice subjected to MS.This study has been supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science.

(COI: No)

S22-3

Maternal behavior of mice which suffered the early-life stress

Takatsuru, Yusuke (*Dept. Integrative Physiol., Gunma Univ. Grad. Sch. Med., Japan*)

Maturation of the brain function still continues during lactating in mammal including human. Thus, relationship between mother and neonate is important not only nutrition supply but also mental stability. The early-life stress during the lactation induces severe effect on brain function and it induces several disorders such as depression. This stress induces instability of synapse and disruption of glutamate homeostasis in brain not only in emotion-rerated area such as hippocampus and prefrontal cortex but also basic area such as somatosensory cortex (Takatsuru et al., 2009, Toya et al., 2014). It is also said that abused children tend to abuse their children when they become to be a mother. This sad phenomena possibly induced by dysfunction of brain which induced by early-life stress. However, underling mechanisms are not still fully understood. We recently showed that the maternal behavior is disrupted in maternal deprivation (MD) mice, one of the early-life stressed models. The success rate of mating decreased in MD mice and even if they became to be a mother, the rate of neglect was also increased. Sadly and interestingly, these changes were also detected in second generation mice. They also showed difficulty in mating and maternal behavior. These changes were detected without early-life stress application to 2nd generation. In conclusion, early-life stress affected the maternal behavior beyond the generation and this MD mouse is potentially good model for study on mechanisms underlying the effect of early-life stress on brain function of human patient case.

(COI: No)

S22-4

Effects of mental task and daily eating behavior on preference to fat and sweet taste in young females

Someya, Nami (*Chiba Prefectural University of Health Sciences, Chiba, Japan*)

It was reported that restrained eaters who intentionally restrict food intake to maintain or lose weight consumed more energy under stressful condition. The purpose of the present study was to evaluate an effect of acute mental task on preference to fat and sweet taste in high and low restrained eaters. Seventeen females (18-22 yrs) participated in the study. The participants were assigned to two groups based on the score of restrained eating scale of the Japanese version of the Dutch Eating Behavior Questionnaire, i.e., high restraint (HR, n=8) and low restraint (LR, n=9). In both groups, Stroop color-word test (CWT) was performed as a mental task for 10 min. Before and after the CWT, the preference to 2, 4, 8, 12, 16 and 20 % fat milk and 25, 50, 100, 150, 200 and 250 mmol/l sucrose solutions were assessed by visual analog scale. In the LR group, the preference rating for milk was peaked at 8 % fat before the CWT, while it was peaked at 12 % fat after the CWT. The preference rating for 12 % fat after the CWT tended to be greater than that before the CWT. On the other hand, there was no significant effect on fat preference in the HR group. In the HR group, the preference rating for sweet taste was peaked at 150 mmol/l sucrose before the CWT, while it was peaked at 200 mmol/l sucrose after the CWT. The preference rating for 200 mmol/l sucrose after the CWT tended to be greater than that before the CWT. There was no significant effect on sweet taste preference in the LR group. These results imply that preference to fat and sweet taste was affected by both mental task and daily eating behavior.

(COI: No)

Symposium 23

Possibility of Joint Lectures and Practicals on Central Nervous System Anatomy and Physiology

(March 21, 17:00～18:30, Room D)

S23-1

Lectures and Practical training of the Central Nervous System from an Anatomical View

Yoshimura, Ken (*Dept. of Anatomy, The Nippon Dental Univ. Sch. of Life Dent. at Niigata*)

We are providing lectures on the central nervous system (CNS) for students in dental faculty. The lecture of CNS from an anatomical view are as follows: (1) The Development of the CNS and the Cerebral Ventricle, (2) The Basic Structure of the Brain and Cerebral Association Fibers, (3) Typical Functional Localizations, (4) The Layers of the Cerebral Neocortex and Associating Pyramidal Cell like Betz, (5) Association areas of the Cerebral Cortex Including the Speech Center like Broca\'s and Wernicke\'s areas, (6) The Basal Ganglia, (7) The Diencephalon, (8) The Brainstem, (9) The Basic Structure of Spinal cord and (10) Several Typical Examples of Conducting Pathways. As well as the above-mentioned morphology-based lecture of the CNS, we also have a practical course. Students observe overviews of the excised whole brain that were covered with the dura mater, arachnoid and pia mater, and then carefully excised from its brainstem, then the brain was sliced. After the observations, students made correlating drawings.Most students seem to be interested in morphology in the CNS. However, they have difficulty understanding it because the CNS lacks morphological characteristics and structures which can be easily assume unlike other organs such as muscles.Therefore, we are providing lectures not only a knowledge-based style but also associating historical information and updates. I would like to introduce the overviews of our anatomical view in lectures.

(COI: No)

S23-2

Current education of practical training and lecture in the central nervous system at physiology side

Satoh, Yoshihide (*Dept Physiol, Sch Dent Niigata, Nippon Dent Univ, Niigata, Japan*)

Students of dental university must learn not only maxillofacial structure including teeth but also function of orofacial and tongue in basic dental science. For example, there are neural mechanisms of orofacial somatic sensation, gustation, mastication, jaw reflexes, swallowing, salivation, articulation and respiration. It is very important for dental students to understand neural pathways and reflex arcs. In the central nervous system, there are many sites that are related to them as follows; the main sensory trigeminal nucleus, the spinal trigeminal nucleus, especially the subnucleus caudalis, the supratrigeminal nucleus, the motor trigeminal nucleus, the facial nucleus, the hypoglossal nucleus, the nucleus of the solitary tract related to the swallowing and the gustation, the superior and inferior salivatory nuclei, the ventral posteromedial nucleus of thalamus, the lateral hypothalamic area as a feeding center, the primary motor cortex, the primary somatosensory cortex, the primary gustatory cortex and so on. However, understanding three-dimensionally of location of these sites is very difficult for dental students, because the structure of the central nervous system is very complicated. An anatomical knowledge is absolutely necessary for all lectures of physiology. On the other hand, physiology practical training about the central nervous system has not done in our university. In this symposium, I would like to talk about challenges of lecture of the central nervous system from the standpoint of physiology.

(COI: No)

S23-3

A possiblity of joint lectures and practicals for anatomy and physiology on the central nervous system

Kageyama, Ikuo (*The NDU, Niigata, Japan*)

A recent survey revealed that the time allotted for lectures in anatomy and physiology is drastically decreasing in medical and dental schools because students are required to study an increasing number of subjects. However, knowledge of anatomy and physiology remains fundamental and essential, especially for clinicians. No one would want a surgeon who lacks knowledge of anatomy and physiology. Student physicians and dentists should learn anatomy and physiology because it is critical for them and their colleagues in co-medical disciplines. The use of joint lectures and practicals might be an efficient method for such study. In this symposium, we will discuss the possibility of joint lectures and practicals on the anatomy and physiology of the central nervous system.

(COI: No)

S23-4

Current Trends in Teaching Neuroanatomy in Sri Lanka

Nanayakkara, Chinthani D. (*Department of Basic Sciences, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka*)

Human anatomy, which includes gross and neuroanatomy, represents a crucial component among the basic sciences providing relevant medical awareness to students in health sciences. The anatomic background is deemed a cornerstone of medical learning for centuries. Likewise, neuroanatomy is considered as the cornerstone in understanding the nervous system and its disorders. Neuroanatomy poses many challenges to students not only because of its numerous discrete structures, but also due to the difficulty in comprehension of topography and complicated spatial relations between them. Traditional undergraduate teaching in neuroanatomy comprise of a group lecture, a gross dissection as well as self study with 2 dimensional (2D) images in atlases. Over the years the amount of time dedicated to anatomy, and therefore to neuroanatomy, also has significantly decreased. Most institutions have developed curricula to overcome many of these constraints by integrating anatomy teaching with physiology and clinical material, and adopting innovative teaching methods using electronic tools providing three-dimensional information. Developing a module on the Nervous System in the new Basic Sciences Curriculum in our institution, and how neuroanatomy and neurophysiology teaching is conducted to increase the clinical relevance and self-directed learning will be discussed.

(COI: No)

S23-5

Current Status and Future Challenges in Teaching Neurophysiology in Sri Lanka

Pallegama, Ranjith W. (*Division of Physiology, Department of Basic Sciences, Faculty of Dental Sciences, University of Peradeniya, Peradeniya 20400, Sri Lanka*)

The development, organization, structure and the general functions of the nervous system are taught in an integrated course. The teaching is done mainly through lectures, practical classes and tutorials; none of the sessions includes combined content in anatomy and physiology. The contents include, organization and function of the peripheral nerves, physiology of somatic sensations including pain, autonomic nervous function, reflexes and their supraspinal control, cortical, cerebellar and brain stem control of motor functions and a limited coverage on higher intellectual functions. The neurophysiology of mastication, swallowing, speech, and special senses are taught in two other separate courses with the relevant anatomy content. Clinical relevance of the content is shown throughout the course. The students practice on clinical examination of the nervous system in practical classes and they study around problems during tutorial classes.Increasing demand for resources and time allocation in curricula by expanding clinical sciences forces the basic medical sciences to shrink the contents. A constructively aligned outcome based education is promoted in Sri Lanka as a general policy in education. The challenges in teaching preclinical sciences in an integrated fashion aiming at the intended outcomes and the potential teaching-learning activities are discussed in this discourse. Qualitative feedback of students indicates their perceptions and is also useful in shaping the future directions in teaching neurophysiology integrated with anatomy.

(COI: No)

Symposium 24

A new vista of study on formation and function of lymphatic vessels

(March 21, 17:00～18:30, Room E)

S24-1

Morphogenetic mechanisms of the lymphatic endothelial cells in zebrafish and Medaka

Isogai, Sumio (*Dept.Anat. Sch.Med.Iwate Med. Uni., Morioka, Japan*)

Despite its importance, lymphangiogenesis had remained poorly understood because the lack of specific markers to identify the lymph vessels and of a small animal model to be genetically manipulated. We have worked to make the zebrafish and medaka new genetic models to unravel the function of candidate gene . We demonstrated that they possess a lymphatic vascular system that shares the morphological, molecular and functional characteristics of the lymphatic vessels in other vertebrates. The main function of the lymph vascular system is to return excess interstitial fluid back to the blood vascular system. In early developmental stages, multiphoton time-lapse imaging of transgenic-fish enabled us to visualize and trace the formation of the thoracic duct and the lymph sac at the jugular angle by following the migration of individual cells from their origin through their incorporation into lymphatic endothelium, which allowed us to definitively determine the ontogeny of this system. The conserved anatomical pattern allows this collecting system to function properly. Some cues and mechanisms guiding the lymph progenitor cells to the gross anatomical pattern were revealed. Using time-lapse imaging and various injection methods, we are investigating the collecting system formation in zebrafish and medaka, and are compiling the anatomical atlas of lymph vascular system from embryo through adult. Our studies provide the detailed morphogenetic characterization of the lymphatic endothelial cells in these organisms, as well as the definitive in vivo evidence for the mechanism underlying the formation of the vertebrate lymphatic system.

(COI: No)

S24-2

Engineering of three-dimensional tissues with blood and lymphatic vascular tubules fabricated by cell-accumulation technique

Asano, Yoshiya[1]; Matsusaki, Michiya[3]; Nishiguchi, Akihiro[3]; Okano, Daisuke[1]; Akashi, Mitsuru[3]; Shimoda, Hiroshi[1,2] (*[1]Dept.Neuroanat.CellBiol.Histl., Grad.Sch. Med.Hirosaki Univ., Hirosaki, Japan; [2]Dept.Anat.Sci., Grad.Sch.Med.Hirosaki Univ., Hirosaki, Japan; [3]Dept.Appl.Chem., Grad.Sch.Eng.Osaka Univ., Suita, Japan*)

Engineering of culture tissue with blood and lymphatic vascular networks, constructed by human cells, can be applied for biomaterials in medical treatment and/or for valuable tools in biomedical researches. We have established three-dimensional tissue-constructing technique named cell-accumulation method, which is based on coating of culture cells by extracellular matrix-nanofilms. By using this method, we have successfully fabricated the artificial tissues with tubular networks of human umbilical venous endothelial cells (HUVECs) and/or human dermal lymphatic endothelial cells (HDLECs) in multilayerd normal human dermal fibroblasts (NHDFs). In ultrastructural analysis, HUVECs and HDLEC formed luminal structures mimicking native blood and lymphatic capillaries, respectively, in connective tissue-like constructs by NHDFs. The artificial lymphatic capillaries showed irregular shapes, loose adhesive connections, and gap formations between endothelial cells, in comparison to artificial blood capillaries. By using our artificial tissues, we demonstrate 1) morphogenetic analysis of blood and lymphatic vascular networks, 2) transplantation of blood or lymphatic vascular tissues as biomedical grafts, and 3) establishment of human peritoneum model with vascular networks for researches of cancer metastasis.

(COI: No)

S24-3

Key roles of lymph flow in the lymphatic function

Kawai, Yoshiko[1]; Ohhashi, Toshio[2] (*[1]Dept. of Physiol., Shinshu Univ. Sch. of Med., Matsumogo, Japan; [2]Dept. of innovation of medical and health sciences research, Shinshu Univ. Sch. of Med., Matsumoto, Japan*)

To address physiological roles of shear stress produced by lymph flow in gene and protein expression in lymphatic endothelial cells with special reference to physiological function of collecting lymph vessels, we firstly investigated the effects of shear stress stimulation on ecNOS expression in cultured lymphatic endothelial cells (LEC). These results suggest that shear stress produces a significant release of ATP from LEC, which activates the purinergic P2X/2Y receptor, thereby facilitating ecNOS mRNA and protein expression through inositol 1, 4, 5-trisphosphate-mediated release of intracellular Ca^{2+} ions and the activation of Ca^{2+}-activated K^+ channels in LEC. We also investigated the effects of shear stress stimulation on release of ATP through activation of F_1/F_0 ATP synthase in the cultured cells, and the mechanisms of shear stress-mediated F_1/F_0 ATP synthase-dependent release of ATP in the cultured cells. Next, we investigated the effects of shear stress stimulation on ICAM-1 expression in cultured LEC. We studied whether shear stress-mediated adhesion molecule expression accelerates the attachment of carcinoma cells to human LEC. Finally, in in vivo experiments we evaluated whether exogenous ATP facilitates the expression of carcinoma cell-ligated adhesion molecules in rat lymph node. In conclusion, shear stress stimulation induces ICAM-1 expression on LEC by activating cell surface F_1/F_0 ATP synthase, which might contribute, in part, to the creation of a premetastatic environment within sentinel lymph node.

(COI: No)

S24-4

Roles of signal networks during the formation of lymphatic vessels

Watabe, Tetsuro[1]; Yoshimatsu, Yasuhiro[1]; Miyazono, Kohei[2] (*[1]Lab. Oncology, Sch. Life Sci., Tokyo Univ. Pharm. Life Sci., Japan; [2]Dept. Mol. Pathol., Grad. Sch. Med., Univ. Tokyo, Japan*)

Members of bone morphogenetic protein (BMP) family have been implicated in the formation of blood vessels. We have previously reported that BMP-9/ALK-1 signals enhance the proliferation of blood vascular endothelial cells (BECs). However, the roles of BMP-9/ALK-1 signals in lymphatic vessel formation remain largely unknown. Here we examined the effects of BMP-9/ALK-1 signals on lymphangiogenesis both in vitro and in vivo. BMP-9 significantly inhibited the proliferation of human dermal lymphatic endothelial cells (HDLECs) in vitro. Importantly, we found that BMP-9 decreased the expression of Prox1, a transcription factor critical for the differentiation and maintenance of lymphatic endothelial cells, concomitantly with decreased expression of lymphatic-specific genes such as VEGFR3 and with increased expression of blood vascular-specific genes such as VEGFR2. In order to study the in vivo functions of BMP-9, we used multiple types of human cancer xenograft models. We observed the decreased formation of lymphatic vessels in tumors derived from tumor cells lentivirally transduced with BMP-9 as compared with those from control tumor cells. Furthermore, we observed that BMP-9 inhibited inflammation-induced lymphangiogegensis in the corneas and diaphragms of immunocompetent mice. Taken together, these results suggest that BMP-9 inhibits lymphangiogenesis both in vitro and in vivo through down-regulation of Prox1 expression, which leads to reprogramming of LECs to obtain BEC phenotypes.

(COI: No)

S24-5

Individualized minimally invasive treatment based on sentinel node concept for early gastric cancer

Takeuchi, Hiroya; Kitagawa, Yuko (*Dept. Surg. Keio Univ. Sch. Med, Tokyo, Japan*)

Clinical application of sentinel node (SN) mapping for early gastric cancer had been controversial for years. However, single institutional results of SN mapping for these cancers are almost acceptable in terms of detection rate and accuracy to determine lymph node status. SN mapping may play a key role to obtain individual metastatic information and allows modification of the surgical procedures for early gastric cancer.The Japan Society of Sentinel Node Navigation Surgery conducted a prospective multicenter trial of SN mapping for early gastric cancer by a dual tracer method with radioactive colloid and blue dye. SN mapping had been performed for 397 patients with early gastric cancer at 12 comprehensive hospitals including our institution. As results, detection rate of hot and/or blue node was 98%. The sensitivity to detect metastasis based on SN status was 93%, and accuracy of metastatic status based on SN was 99%. Based on these results, minimized gastrectomy such as partial gastrectomy, proximal gastrectomy, segmental gastrectomy and pylorus-preserving gastrectomy with individualized selective and modified lymphadenectomy for early gastric cancer with negative SN has been performed in our institution. More recently the combination of endoscopic resection with SN biopsy also appears attractively.Sensitivity of intraoperative diagnosis of micrometastasis is crucial for SN mapping in gastric cancer. However, the clinical impact of micrometastasis or isolated tumor cells detected by histopathology and molecular analysis in SNs of patients with early gastric cancer remains controversial.

(COI: No)

Symposium 25

Auditory information processing in local ciruit of the inferior colliculus

(March 21, 17:00～18:30, Room G)

S25-1

Organization of local circuit in the inferior colliculus

Ito, Tetsufumi (*Faculty Med. Sci. Univ. Fukui, Fukui, Japan*)

The inferior colliculus (IC) receives ascending inputs from virtually all lower brainstem auditory nuclei in which auditory information is extracted in parallel and integrate the sound information to create de novo responses to features of sound. However, morphological evidence for the integration of parallel auditory streams remained unclear. Large GABAergic (LG) cells are tectothalamic inhibitory neurons and characterized by the dense excitatory axosomatic terminals. We hypothesized that dense excitatory axosomatic terminals on LG cells arise from both local and ascending neurons. Injection of Sindbis palGFP virus in auditory brainstem nuclei resulted Golgi-like labeling of neurons at a single axonal level. A single excitatory axon from the lower brainstem auditory nuclei and IC made 1-6 axosomatic contacts on an LG cell, suggesting convergence of inputs from many neurons. These inputs are unlikely to be from a single brainstem nucleus since lesions of single nuclei fail to eliminate most excitatory axosomatic terminals on the LG cells. A single local excitatory neuron made laminar axonal plexus and contacted on cell bodies of 10-30 LG cells, suggesting that activation of a local neuron elicits synchronized excitation of LG cells in the same laminae. Double injections of different viruses in IC and in a brainstem nucleus showed that LG cells received inputs from both. The LG cell bodies in different IC regions received inputs from different brainstem nuclei, suggesting that combination of ascending inputs may different between LG cells in different locations. Such neural circuitry may underlie the integration of auditory information on a single IC neuron.

(COI: No)

S25-2

A temporal integration mechanism enhances frequency selectivity of broadband inputs to inferior colliculus

Chen, Chen[1]; Read, Heather L[2,3]; Escabi, Monty A[1,2,3] ([1]*Electrical and Computer Engineering, UConn, USA;* [2]*Biomedical Engineering, UConn, USA;* [3]*Psychology, UConn, USA*)

Mammals can accurately resolve frequency components in sounds, a task that is essential for sound recognition. Despite its importance, there is little direct evidence for how frequency selectivity is preserved or newly created across auditory structures. Here we demonstrate that pre potentials with properties resembling broadly tuned brainstem inputs can be recorded concurrently with post synaptic action potentials in inferior colliculus (IC). These putative input neurons (PIN) are broadly tuned and exhibit temporally delayed and spectrally interleaved excitation and inhibition not present in the paired IC neurons (ICN). A radical sharpening of tuning is accomplished locally by temporal integration of the broad converging inputs. A neuron model replicates the finding and demonstrates that temporal integration degrades timing precision but enhances spectral selectivity through interference of spectrally in- and out-phase inputs. This contrasts current models that require local inhibition to enhance selectivity and supports an alternative computational strategy to quickly enhance frequency selectivity.

(COI: No)

S25-3

Deviance detection in the auditory brain

Ayala, Yaneri A[1]; Oliver, Douglas L[2]; Malmierca, Manuel S[1] ([1]*Institute of Neuroscience of Castilla y Leon, University of Salamanca. Spain;* [2]*University of Connecticut Health Center, USA*)

The ability to discriminate novel stimuli is important for survival and it requires neuronal mechanisms to extract relevant information, to code regularities and to detect changes occurring in the acoustic scene. Neurons that exhibit stimulus-specific adaptation (SSA) may be involved in these computational tasks. SSA is a form of neural habituation in which the neuronal response to a common sound is diminished but not generalized to other sounds that occur rarely. We studied the anatomical and physiological correlates of SSA exhibited by single neurons in the inferior colliculus of the anesthetized rat. Our results suggest that the inferior colliculus is the first auditory stage where SSA emerges, and that neurons with strong SSA responses are located in the non-lemniscal subdivisions. Moreover, our studies indicate that the inputs to SSA recording sites differ from those to the non-SSA recording sites. This suggests specific synaptic domains provide inputs to neurons sensitive to deviant sounds. Finally, we demonstrate in iontophoretic studies that inhibitory and cholinergic inputs modulated SSA differently. The blockade of inhibition increased the response to common and deviant sounds while the acetylcholine only increased the response to the common sound. These data describe features of the neuromodulation and connectivity of a distributed neural network for mapping the saliency of the auditory scene.

(COI: No)

S25-4

Excitatory and inhibitory synaptic interactions underlying binaural hearing in inferior colliculus neurons

Ono, Munenori; Oliver, Douglas L (*Dept Neurosci, University of Connecticut Health Center, USA*)

Localizing the sound source by binaural hearing is critical for animal to survive. The inferior colliculus (IC) is a pivotal auditory center in the midbrain, yet little is known about the synaptic mechanisms that underlie binaural hearing in the inferior colliculus (IC). Here, we study the synaptic currents that process binaural hearing in vivo by voltage clamp technique. Monaural stimulation in either ear produced EPSCs and IPSCs in most neurons. The temporal properties of monaural responses were well matched, suggesting connected functional zones with matched inputs. Further, we studied the responses to interaural level difference (ILD), an important sound localization cue, by using stimuli in which ILD varies around a constant average binaural level to approximate sounds on the horizontal plane. EPSCs and IPSCs were well correlated in the response to the stimuli with different ILDs. Summation of the monaural EPSCs predicted the binaural excitatory response but less well than the summation of monaural IPSCs. Binaural EPSCs often showed a nonlinearity that strengthened the response to specific ILDs. Extracellular spike and intracellular current recordings from the same neuron showed that the ILD tuning of the spikes was sharper than that of the EPSCs. Thus, in the IC, balanced excitatory and inhibitory inputs maybe a critical feature of synaptic coding in sound localization.

(COI: No)

S25-5

In vivo optical and electrical recordings from inferior colliculus neurons by micro-endoscope

Funabiki, Kazuo[1,2,3]; Yashiro, Hidetaka[2]; Nakahara, Ichiro[1,3]; Kobayashi, Kohta[2]; Riquimaroux, Hiroshi[2] ([1]*OBI, Systems Biology, Japan;* [2]*Doshisha Univ. Life Med. Sci., Japan;* [3]*Kyoto Univ. Biostudies, Japan*)

In vivo Ca2+ imaging is a powerful method for the functional assessment of neural circuits. Although multi-photon excitation and two-photon fluorescence microscopy are used widely, observation of circuits in deep brain regions remains challenging. Recently, observing these deep regions has become possible via an endoscope consisting of an optical fiber bundle or gradient-index lens. We have developed a micro-endoscope system that enables simultaneous optical recording of fluorescence and electrical recording of neural activity. Using this system, we recorded auditory responses by simultaneously detecting changes in the fluorescence intensity of a Ca^{2+} indicator dye, multiunit activities, and local field potentials in the mouse's inferior colliculus (IC). Optical and electrical recording methods supplemented each other by providing high-resolution spatial and temporal information, respectively. By systematically changing sound frequency and intensity, we determined the frequency tuning of the recording site. The best frequency shifted higher as the probe advanced more deeply, demonstrating that the system is capable of optically measuring the dorsoventral organization of IC (i.e., tonotopicity). Thus, our new micro-endoscope system will be useful in analyzing neural circuits, including those within the auditory system.(CIO:No)

(COI: No)

Symposium 26

Clinical needs and Clinical anatomic researches

(March 21, 17:00～18:30, Room H)

S26-1

The extension pattern of a deep anal fistula in comparison with three-dimensional structures of the anal sphincter muscles and ischiorectal fossa

Kagawa, Ryuzaburo (*Rakuwakai Otowa Hosp, Kyoto, Japan*)

The primary abscesses of most deep anal fistulas are present in the posterior intersphincteric space at level of the deep part of external anal sphincter, or in the adjacent damaged external anal sphincter. From this origin, the tract of the fistula extends according to the three-dimensional structure of the anal sphincter muscles and ischiorectal fossa.
The bilateral puborectal muscles, in their anterior and posterior parts, send muscle fibers to the upper areas of the deep parts of the external anal sphincter muscles and form a ring-shaped muscle in a deep part of the external anal sphincter muscle. In ischiorectal anal fistula, the primary abscess, formed at the level of the deep part of external anal sphincter, penetrates the sphincter muscle in the directions of 5 and 7 o'clock and forms abscesses in the ischiorectal fossa below the levator ani muscle, following the course of the confluent puborectal muscle fibers.
In the adipose tissue of the ischiorectal fossa, there is a septum surrounding the anal sphincter muscles from the anteroposterior and vertical sides. An abscess that has penetrated the deep part of the external anal sphincter muscle in its posterior part extends along this septum. This results in the characteristic morphology of anal fistulas of the ischiorectal fossa with flat tracts advancing anteroinferiorly in the ischiorectal fossa.
In this report, I show jack-knife position MRI that three-dimensional analysis is possible with the structure of all deep anal fistulas.

(COI: No)

S26-2

My fellow anatomists, ask not what the physicians can do for you, ask what you can do for the physicians

Ibukuro, Kenji (*Diag Rad, Mitsui Mem Hosp, Tokyo, Japan*)

When I was a medical student, one of the first things we learned was the cutaneous distribution of the nerve in the upper arm. When I became a resident in radiology, I was asked to puncture the vein for injection of the contrast media. However, I noticed that I did not know much about the relation between the cutaneous nerve and the vein of the upper arm, when the patient complained the pain. The MHLW allowed the nurses to puncture the vein and place the indwelling needle several years ago. So we radiologist asked them to do that, but I was asked to tell them how to do it safely. I tried to gather the anatomical information about it, but I found very few. I thought what I could do was to dissect the cadaver by myself and show the nurses the result. Although the venous puncture is a simple procedure, it is undeniable fact that some patients sued the health care provider for the contiguous pain, peripheral nerve paralysis, etc. So how do we avoid these troubles?When it is necessary to place the central venous catheter, we should puncture either the subclavian or internal jugular vein. Compared with the peripheral venous puncture mentioned above, the complications related to this procedure are sometimes fatal. We need to know at least the venous anatomy but the anatomical knowledge with surrounding structure is more important to prevent or less the complications. So how do we get the anatomical knowledge in detail?If the anatomist would like to remain in the faculty of "medicine", you should participate to solve these clinical problems and "present danger", otherwise, you should be transferred to anthropology.

(COI: No)

S26-3

Anatomy for the clinicians (especially for the surgeons) based on my experience as a surgeon

Watanabe, Koichi; Iwanaga, Joe; Tabira, Yoko; Saga, Tsuyoshi; Yamaki, Koh-ichi (*Kurume Univ. Sch.Med., Fukuoka, Japan*)

The presenter is in the anatomy department now, but used to be engaged in plastic surgery over fifteen years. In this presentation, I would like to report what clinicians require to the anatomy with the based on my personal experience and my colleagues'(both clinicians and anatomists) opinion. I believe anatomical knowledge is extremely important for surgeons of every levels from resident to operator. In the case of residents, over five years have passed after anatomical lectures in the school. I consider that two problems are involved in them. One is that they usually forget majority of anatomical knowledge which the learned. And another is that the knowledge of regional anatomy which is not taught fully in the school is suddenly required in the surgery. To teach accurate anatomical knowledge is considered to be extremely important for the surgical training. The full-trained surgeons usually have a wide variety of anatomical knowledge about the region they usually treat. However, they also become to meed new anatomical knowledge in the case they have to perform inexperienced or newly developed operations. Furthermore, they need anatomical knowledge when they develop new operations or improve previous operations. In our department, we often collaborate with other clinical departments about the anatomical research and post graduate education. I consider that anatomists cooperate with clinicians more than ever and provide the anatomical knowledge in accordance with the level of surgeons. It will be benefit for both patients and clinicians.

(COI: No)

Symposium 27

Relationship between cellular functions and membrane transporters/ion channels

(March 21, 17:00～18:30, Room I)

S27-1

Removal of uremic toxin amelioreates the down regulation of SLCO4C1 transporter through transcriptional pathway

Abe, Takaaki (*Tohoku University Graduate School of Biomedical Engineering and Tohoku University Graduate School of Medicine, Japan*)

The accumulated uremic toxins inhibit the expression of various renal transporters and this inhibition may further reduce renal function and subsequently cause the accumulation of uremic toxins. However, the precise mechanism of the nephrotoxicity of uremic toxins on renal transport has been poorly understood. Recently, we have report that indoxyl sulfate, one of the potent uremic toxins, directly suppresses the renal-specific organic anion transporter SLCO4C1 expression through a transcription factor GATA3. The promoter region of SLCO4C1 gene has several GATA motifs, and indoxyl sulfate up-regulated GATA3 mRNA and subsequently down-regulated SLCO4C1 mRNA. Overexpression of GATA3 significantly reduced SLCO4C1 expression, and silencing of GATA3 increased SLCO4C1 expression vice versa. Administration of indoxyl sulfate to rats reduced renal expression of slco4c1 and under this condition, plasma level of guanidinosuccinate, one of the preferable substrates of slco4c1, was significantly increased without changing plasma creatinine.
Furthermore, in 5/6 nephrectomized rats, treatment with oral adsorbent AST-120 significantly decreased plasma indoxyl sulfate level and conversely increased the expression of slco4c1, following the reduction of plasma level of guanidinosuccinate. These data suggest that the removal of indoxyl sulfate and blocking its signal pathway may help to restore the SLCO4C1-mediated renal excretion of uremic toxins in CKD.

(COI: No)

S27-2

Characterization of LAT1 as a central transporter of essential amino acids in activated human T cells

Hayashi, Keitaro; Jutabha, Promsuk; Anzai, Naohiko (*Dept Pharmacol and Toxicol, Dokkyo Med Univ Sch Med, Tochigi, Japan*)

Activation of T cells accompanies remarkable enhancement of metabolism. Sufficient and continuous supply of nutrient such as amino acids is thus considerable importance to support immune reaction in T cells. However, the molecular mechanism of efficient incorporation of amino acids into activated T cells has not been determined. We characterized L-type amino acid transporter 1 (LAT1) as an essential amino acids transporter in activated human T cells. The activation of primary human T cells by CD3/CD28 stimulation triggers the dramatic induction of LAT1 mediated by NF-kB and AP-1. JPH203, a specific inhibitor of LAT1 suppressed amino acid incorporation and induction of DNA-damage-inducible transcript 3 (DDIT3) to attenuate cytokine production via inhibition of NF-kB and NFAT activities in activated human T cells. These results indicate that LAT1 expression is induced by full activation of T cells and works as a central transporter for essential amino acids in activated T cells. Our result uncover the previously unknown mechanism by which T cells accelerate essential amino acid uptake upon activation and adapt to essential amino acid starvation.

(COI: No)

S27-3

Localization of ATP sensitive K+ channel subunits in different organs and their possible functions

Zhou, Ming[1]; Kawahara, Katsumasa[2]; Abe, Hiroshi[1] ([1]*Akita Univ.Grad.Sch.Med., Akita, Japan;* [2]*Kitasato Univ.Sch.Med., Sagamihara Japan*)

ATP sensitive K+ (KATP) channel originally discovered in cardiomyocytes. From then, several kinds of KATP channel subunits were found. KATP channel has specific characteristics of channel opening and closing controlled by changes of intracellular ATP in micromole concentrations. It was considered with important functions of insulin secretion, cell protection against cardiac ischemia or brain anoxia. In cellular localization, it was proved in pancreatic β-cells, in cardiomyocytes, and in neurons and glia. KATP channels are composed of pore-forming subunits, which allow the potassium ion passing through, and regulatory subunits controlling the channel activity. The pore-forming subunits are Kir6.1 and Kir6.2, belonging to inwardly rectifying K+ channel subfamily, well, the regulatory subunits are SURs (SUR1, SUR2A and SUR2B), belonging to ATP binding cassette superfamily. Recently, a prior study of our lab (Zhou et al., Neurosci Res 2012) showed KATP channel subunits SUR2A and SUR2B differently localized in neurons and glial cells. The SUR2A localized mainly in neurons and in some oligodendrocytes, well the SUR2B weakly in neurons but mainly in astrocytes and some oligodendrocytes. The results from our lab also revealed that KATP channel subunits localized in other organs such as heart, kidney, submandibular gland, testis, ovary and pituitary gland. The wide and differential distribution of KATP channel subunits in those target cells and tissues indicates their diversity of relationship between biomedical metabolism and related possible functions.

(COI: No)

S27-4

Water and electrolytes transport across kidney collecting ducts through vasopressin receptors

Kawahara, Katsumasa[1]; Yasuoka, Yukiko[1]; Nonoguchi, Hiroshi[2] ([1]*Dept Physiol, Kitasato Univ Sch Med, Sagamihara, Japan;* [2]*Kitasato University Medical Center, Kitamoto, Japan*)

Vasopressin V1a receptor (V1aR) is known to modulate luminal water permeability of kidney collecting duct (CD) principal cells (PC) by competing with signals of V2R-cAMP-AQP2 axis, however, localization and role of V1aR in the CD are still controversy. In wild-type (WT) and V1aR knockout (KO) mice, we examined localization and expression of V1aR mRNA along the kidney nephron under the conditions of control (normal) and chronic metabolic acidosis (CMA). In control animals, normalized levels of the V1aR mRNA expression were high in macula densa and CD, low in glomerulus (Glm), thick ascending limb of Henle loop (TAL), and distal convoluted tubule (DCT). Surprisingly, we found that in CD, V1aR mRNA expressed in type A and type B intercalated cells (IC-A and IC-B, respectively). Under NH4Cl load, the level of V1aR mRNA increased only at medullary TALis and outer medullary CDis (is: inner stripe). We also found that in KO mice, lower urine concentration ability and metabolic acidosis vs. WT mice. In conclusion, V1aR may play an important role for controlling the basal water permeability in PC and stimulating urinary acid excretion by IC-A.

(COI: No)

S27-5

Physiological Significance of Delayed Rectifier K+-Channels (Kv1.3) Expressed in T lymphocytes and Their Pathological Significance in Chronic Kidney Disease

Kazama, Itsuro (*Dept Physiol I, Grad Sch Med, Tohoku Univ, Sendai, Japan*)

T lymphocytes predominantly express delayed rectifier K+-channels (Kv1.3) in their plasma membranes. Patch-clamp studies revealed that the channels play crucial roles in facilitating calcium influx necessary to trigger the lymphocyte activation and proliferation. In addition to selective channel inhibitors that have been developed, we recently showed physiological evidence that the drugs, such as non-steroidal anti-inflammatory drugs, antibiotics, anti-hypertensives and anti-cholesterol drugs, effectively suppress the channel currents in lymphocytes, and thus exert immunosuppressive effects.Using experimental animal models, previous studies revealed the pathological relevance between the expression of ion channels and the progression of renal diseases. As an extension, we recently demonstrated that the overexpression of lymphocyte Kv1.3-channels contributed to the progression of chronic kidney disease (CKD) by promoting cellular proliferation and interstitial fibrosis. In our most recent study, benidipine, a potent dihydropyridine calcium channel blocker which also strongly and persistently inhibited the lymphocyte Kv1.3-channel currents, actually ameliorated the progression of renal fibrosis in rat models with advanced chronic renal failure. Together with our in vitro results, the studies indicated the therapeutic potency of Kv1.3-channel inhibitors in the treatment or the prevention of CKD.

(COI: No)

S27-6

Therapeutic implications of myofibroblast TRP channels for stenotic fibrosis in Crohns disease

Kurahara, Lin[1]; Hiraishi, Keizo[1]; Sumiyoshi, Miho[1]; Aoyagi, Kunihiko[2]; Inoue, Ryuji[1] ([1]*Dept Physiol, Sch Med, Fukuoka Univ, Fukuoka, Japan;* [2]*Dept Gastroenterol, Sch Med, Fukuoka Univ, Fukuoka, Japan*)

Intestinal fibrosis is a frequent complication of Crohns disease (CD) and often leads to detrimental stricture formation. Myofibroblasts play active roles in mediating fibrotic changes in various tissues. In this study, we investigated whether transient receptor potential (TRP) channels in myofibroblasts are involved in CD-associated intestinal fibrosis, for the purpose of exploring its possible therapeutic targets.A profibrotic factor TGF-β1 treatment transformed spindle-shaped InMyoFibs into filament-shaped cells with enhanced α-SMA, N-cadherin, TRPC4 and TRPC6 expression. Augmented Ca^{2+} influxes due to TRPC6 upregulation facilitate stress fiber formation and strengthen cell-cell interactions by negatively regulating the synthesis of anti-fibrotic factors IL-10 and IL-11 in TGF-β1-treated myofibroblasts. Similar changes were observed in stenotic areas of CD patients, suggesting the therapeutic significance of targeting TRPC6. Active ingredients of Daikenchuto (TU-100) a traditional oriental herbal medicine used for post-operative ileus and constipation, such as hydroxy α-sanshool and [6]-shogaol induced Ca^{2+} influxes due to TRPA1. 24 hour incubation with TU-100 accelerated the mRNA and protein expression of TRPA1 in InMyoFibs. TU-100 also ameliorated Type I Collagen, α-SMA, N-cadherin expression and the phosphorylation of Smad2 and p38-MAPK at the downstream of TGF-β1.These results suggest that TRPC6 and TRPA1 channels could be promising targets for anti-fibrotic therapies in the gut.

(COI: No)

S27-7

Expression, function and phenotype of CFTR mutants found in Japanese CF patients

Sohma, Yoshiro[1]; Yu, Yingchun[1]; Nakakuki, Miyuki[2]; Ishiguro, Hiroshi[2] ([1]*Dept Pharmacol, Sch Med, Keio Univ, Tokyo, Japan;* [2]*Dept Human Nutrition, Sch. Med. Nagoya Univ. Nagoya, Japan*)

Cystic Fibrosis Transmembrane conductance Regulator (CFTR) functions as an ATP-dependent anion channel after a PKA-dependent phosphorylation. CFTR is expressed primarily in epithelial cells and its dysfunction causes Cystic fibrosis (CF), a life-shortening hereditary disease mainly affecting white Caucasians through insufficient exocrine. On the other hand, we recently reported that CFTR was expressed in pancreatic β-cells and its dysfunction induced a diabetes mellitus [1], which suggested that a group of Japanese diabetes patients might be caused by acquired CFTR dysfunctions. We investigated expression and function of nine CFTR mutants found in Japanese CF patients. In whole cell (WC) clamp experiment, R347H-, T633P- and T1220I-CFTR showed a WC current comparable to WT-CFTR. L441P- and R1066C-CFTR showed a smaller but significant WC current than WT-CFTR, however, no or little currents on Y517H-, E267V, M152R- and T1086I-CFTR. In the western blotting, R347H- and T633P-CFTR showed the mature C band which intensity was higher than the premature B band. R1066C-CFTR showed the B band higher than the C band whereas E267V- and T1086I-CFTR showed minimal signals for both B and C bands. These results are generally consistent with their phenotype.

[1] Guo JH, Sohma Y, *Chan HC. Glucose-induced electrical a ctivities and insulin secretion in pancreatic islet B -cells are modulated by CFTR. Nat Commun. 15;5: 4420, 2014.

The authors have declared no conflicts of interest.

(COI: No)

Symposium 28

New research focuses on the structure and function of gastric parietal cells

(March 21, 17:00～18:30, Room J)

S28-1

New and multiple functions of parietal cells

Ueyama, Takashi (*Wakayama Med.Univ., Wakayama, Japan*)

Gastric parietal cells are well-known in exocrine function of secreting hydrochloric acid into the gastric juice, which is antagonized by proton pump inhibitor (PPI). Here, new functions of parietal cells and PPI are proposed.

First, gastric parietal cells serve an endocrine function, whereby estrogen is synthesized and secreted into the portal vein. Gastric estrogen is not a simple sex steroid specific to female but a steroid common to both sexes. As one possibility, gastric estrogen may act as a local regulator of the gastro-hepatic axis.

Second, gastric hydrochloric acid supports the homeostasis of bone. Gastrectomy (GX) was thought to result in osteomalacia due to deficiencies in Vitamin D and calcium. Using a GX rat model, GX induced high turnover of bone with hyperosteoidosis, prominent increase of mineralization and increased mRNA expression of both osteoclasts and osteoblasts-related genes. The increased 1, 25 $(OH)_2$ D_3 level and unchanged PTH and calcitonin levels suggested that conventional bone and calcium metabolic pathways were not involved or changed in compensation. Gene expression profiles through microarray analysis and data mining using Ingenuity Pathway Analysis indicated 9 genes were hubs connected with tissue development and immunological diseases. These results suggest that chronic systemic inflammation might underlie the GX-induced pathological changes in bone.

Third, lansoprazole, a potent PPI, has an alternative indication in the prevention and treatment of oxidative hepatic damage through the induction of both phase I and phase II drug-metabolizing systems, i.e. the AhR/Cyp1a1/Nrf2 pathway in hepatocytes.

(COI: No)

S28-2

Localization and function of ion-transporting proteins involved in gastric acid secretion

Sakai, Hideki; Fujii, Takuto; Shimizu, Takahiro (*Dept. Pharm. Physiol., Grad. Sch. Med. Pharm. Sci., Univ. Toyama, Toyama, Japan*)

Hydrochloric acid (HCl) secretion by gastric parietal cells is accompanied with dramatic morphological changes. In resting parietal cells, tubulovesicles are present in intracellular compartments underlying the apical membrane. Upon stimulation, the tubulovesicles translocate and connect with the apical membrane, resulting in massive acid secretion. Proton is actively secreted by H, K-ATPase, a gastric proton pump, present in both the tubulovesicular and apical membranes. However, it has not been established what molecules contribute to Cl^- transport for HCl secretion. Here, we focus on two Cl^--transporting molecules (ClC-5, a chloride-proton exchanger and KCC4, a potassium-chloride co-transporter). We found that both ClC-5 and KCC4 are expressed in the parietal cells which secrete actively HCl at the luminal region of gastric glands. ClC-5 was co-immunoprecipitated with H, K-ATPase in tubulovesicles (TV), whereas KCC4 was co-immunoprecipitated with H, K-ATPase in the stimulation-associated vesicles (SAV) derived from the apical membrane. ClC-5 and KCC4 were functionally coupled with H, K-ATPase in TV and SAV, respectively. KCC4 may function as a K^+-supplying molecule for H, K-ATPase and also as a Cl^--transporting molecule for HCl secretion. In the resting phase of the parietal cells, KCC4 and H, K-ATPase in the apical membrane of the parietal cell are the main machineries for the basal gastric acid secretion. In the stimulated phase, they also contribute to acid secretion together with ClC-5 and H, K-ATPase in tubulovesicles.

(COI: No)

S28-3

Visualization of the intracellular signaling that integrates the gastric secretion

Fukushi, Yasuko; Sakurai, Takashi; Terakawa, Susumu (*Medical Photonics Research Center, Hamamatsu Univ. Sch. Medicine, Hamamatsu, Japan*)

The gastric gland consists of many kinds of cells such as parietal cells, chief cells and ECL cells; each secretes HCl, mucus and enzymes, respectively. These cells might cooperate for smooth gastric secretion without leaving any damage to each other. The presence of the gap junctions is known in the gastric gland. These gap junctions, mainly formed by connexin32, might be important for the defense system and for the regulation of acid secretion. However, the actual signal transmission through these gaps has not physiologically been demonstrated in the gastric gland yet. Moreover, there is few report elucidating such an intercellular signaling related to the cooperative secretion. In this study, we demonstrate a fluorescence image showing a histamine-induced intracellular signal that propagates from cell to cell in a living gastric gland isolated from the guinea pig. They were stained with acridine orange (AO), and then stimulated with 100 μM histamine. The green fluorescence of AO in the parietal cells was transiently increased, and the same response propagated along the gland for a long distance over many cells. Moreover, local stimulation with histamine on a couple of cells in the presence of suramin induced the propagation also. However, the fluorescence response was suppressed by addition of H89, an inhibitor of the PKA. It was concluded that the propagation of fluorescent signal along the gland reflects cAMP-dependent intercellular signals and that these signals indicate coordinated activations of a combination of many secretory cells in the gastric gland.

(COI: No)

S28-4

E-cadherin on the parietal cell in Helicobacter pylori infected gastric mucosa

Murakami, Motonobu; Fukuzawa, Mayu (*Doshisha Women's College of Liberal Arts, Kyoto, Japan*)

Atrophic gastritis caused by Helicobacter pylori is characterized by loss of parietal cell, which plays an important role in the maintenance of the normal structure of gastric mucosa. The gastric glandular unit provides a model for studying the processes that occur during deregulation of Helicobacter pylori infected gastric mucosa such as increase of proliferation in the isthmus, dysregulation of differentiation, and induction of cell death. We investigated the effects of gastric Helicobacter pylori infection on E-cadherin on parietal cells, generation of parietal cell, and deregulation of gastric glandular unit in Mongolian gerbils after inoculation with Helicobacter pylori. The number of parietal cells, staining of E-cadherin, and Ki67 in the stomach were investigated by immunohistochemistry with monoclonal antibodys against H+/K+-ATPase, E-cadherin, and Ki67 respectively. After inoculation, E-cadherin and parietal cells are lost and marked increase in proliferative activity of the proliferation zone in the isthmus with no new generation of parietal cells and microcirculatory disturbance of the mucosa were obserbed. Adherens junction plays a crucial role in the integrity of epithelium and E-cadherin is a key molecule in the morphogenesis, regulation of the fate of cells in proliferation and differentiation. Our study strongly suggests that disruption of E-cadherin in the inflamed mucosa plays an important role in the induction of parietal cell loss and deregulation in the gastric isthmus, leading to the gastric mucosal dysorganization observed in atrophic gastritis.

(COI: No)

S28-5

Exfoliation of gastric pit-parietal cells into the gastric lumen associated with autophagic degradation: an in vitro study discovered in iGM model

Toyoshima-Aoyama, Fumiyo; Takahashi, Nobuyasu; Sawaguchi, Akira (*Univ. Miyazaki, Miyazaki, Japan.*)

Parietal cells are produced in the isthmus of the gastric gland and migrate either upward to the pit region (referred to as pit-parietal cell) or downward to the glandular base via the neck region in rodents. Pit-parietal cells are highly differentiated cells responsible for the gastric hydrochloric acid secretion. We have recently noted that a number of pit-parietal cells were exfoliated into the lumen of isolated rat gastric mucosa (iGM) model, and applied the cryotechniques to elucidate the fine structure and histochemical characteristics in the process of cell exfoliation.As results, quantitative analysis clarified a time-dependent increase in the number of cell exfoliation in the iGM under histamine-stimulation compared to acid-inhibition with H_2-antagonist or proton pump inhibitor. Immunohistochemical staining of LC3 and beclin-1 demonstrated positive reactions in the exfoliated pit-parietal cells, suggesting that the cell exfoliation was preceded by autophagic degradation. In addition, immuno-electron microscopy verified characteristics of the autophagic cell death in the exfoliated pit-parietal cells, forming a number of autophagic ultra-structures in the cytoplasm. Another striking finding is that the pit-parietal cell exfoliation was usually accompanied by extension of the cytoplasmic processes from adjacent surface mucous cells. This finding indicates a crucial sealing of the basal region between the exfoliating pit-parietal cell and the basement membrane, to prevent an epithelial deficiency and possible gastric erosion and ulcer.

(COI: No)

Symposium 29

The structural cell physiology of tight junction protein claudin

(March 22, 9:00～10:30, Room C)

S29-1

Role of Tight Junction Claudins in Biological Systems-More than a Simple Paracellular Barrier in Biological Flow-

Tsukita, Sachiko; Tamura, Atsushi (*Grad. Sch. of Front. Biosci. and Grad.Sch.of Med., Osaka Univ., Osaka, Japan*)

Epithelial cell sheets cover the outer and inner surfaces of every compartment in the body, at every level, from the cell to the body surface, and at all of these levels, they function as a permselective barrier. The paracellular barrier, which is established by the formation of tight junctions between epithelial cells, has received considerable attention lately, because of new information about the claudin-family proteins, tight-junctional membrane proteins with four membrane-spanning regions. Claudins play a critical role both in paracellular permselectivity and as a structural component of the paracellular barrier. In this respect, the knockout mouse analyses, in which single claudins or multiple claudins in different combinations are targeted, are beginning to elucidate the in vivo biological relevance of paracellular barriers/permeability in many aspects of biological systems' functioning. In this respect, in these years we revealed the role of ion-leaky claudins claudin-2 and/or 15, which selectively permeate ions such as Na+, in the nutrient absorption systems. In digestive systems, the inflammation system backboned the safety guard for which tight junctional claudins play an important role. Thus, we would like to discuss the tight junction- based construction of biological systems in general.

(COI: No)

S29-2

Crystal structure of a claudin, a main component of tight junctions

Tani, Kazutoshi[1]; Suzuki, Hiroshi[1]; Tamura, Atsushi[2]; Tsukita, Sachiko[2]; Fujiyoshi, Yoshinori[1,3] ([1]*Cellular and Structural Physiology Institute, Nagoya Univ., Nagoya, Japan;* [2]*Laboratory of Biological Science, Graduate School of Frontier Biosciences and Graduate School of Medicine, Osaka Univ., Osaka, Japan;* [3]*Department of Basic Medical Science, Graduate School of Pharmaceutical Science, Nagoya Univ., Nagoya, Japan*)

Tight junctions are cell-cell adhesion structures in epithelial cell sheets that form boundaries to regulate the paracellular permeation of solutes inside and outside of multicellular organisms, and are crucial for maintaining homeostasis. Claudins are the major constituents of the tight junction strands and function as cell adhesion molecules and paracellular barriers. Recently we reported the first crystal structure of a mammalian claudin, revealing a transmembrane four-helix bundle that supports an extracellular domain with a unique β-sheet architecture. It comprises two extracellular segments and is anchored in a crevice between transmembrane helices by the highly conserved W-LW motif. Combined with the results of our mutational experiment, a linear arrangement of claudin-15 monomers in the crystal suggests that inter-molecular interactions for polymerization form potential paracellular ion pathways with distinctive surface charges. Our proposed model explains the dual functions of TJs in forming barriers as well as paracellular channels in epithelial cell sheets. Our findings provide insight into the molecular basis of the structure and function of tight junctions.

(COI: No)

S29-3

Structure and diversity of gap junction channels studied by electron microscopy

Oshima, Atsunori (*CeSPI, Nagoya Univ, Nagoya, Japan*)

Invertebrate-specific gap junction proteins, termed innexins, form a large family of four-transmembrane proteins. These proteins oligomerize to constitute intercellular channels that allow for the passage of small signaling molecules associated with neural and muscular electrical activity in invertebrates. In contrast to the large number of structural and functional studies of vertebrate connexin gap junction channels, there are few structural works on recombinant innexin channels. Here we show an electron microscopic analysis of solubilized and 2D crystallized Caenorhabditis elegans innexin-6 (INX-6) gap junction channels. Negative-staining electron microscopy (EM) of purified INX-6 gap junction channels revealed tandem particles. Class averages from those images indicated a longitudinal height of 220 Å, a channel diameter of 110 Å in the absence of detergent micelles, and an extracellular gap space of 60 Å. Single particle cryo-EM of purified INX-6 channels revealed eight rotational peaks related to the innexin subunit organization. We recently obtained 2D crystals of INX-6 channels, and cryo-EM crystallographic analysis revealed a projection map showing eight clear and separate densities around the pore. Initial 3D reconstruction at 10 Å resolution revealed that a single INX-6 full gap junction channel comprises 16 subunits, a hexadecamer. We also found plug densities in the opposing hemichannels, reminiscent of the Cx26M34A structure we previously reported. These results suggest that the oligomeric number of INX-6 channels is distinct from that of vertebrate connexin channels, and provide insight into innexin channel function.

(COI: No)

S29-4

Correlation of claudin molecular properties with its channel or barrier functions

Fromm, Michael; Krug, Susanne; Milatz, Susanne; Rosenthal, Rita; Piontek, Jorg; Gunzel, Dorothee (*Univ.med.Berlin, Berlin, Germany*)

Claudins connect neighboring epi- and endothelial cells by forming polymers which tighten the tissue layer against paracellular passage of solutes and water. Besides this general function, some claudins provide specific permeation sites. By this they form channels which, unlike common membrane channels, lead through extracellularly located pores. Several channel-forming claudins have been identified so far, some of them being selective for cations (claudin-2, -10b, -15), others for anions (claudin-10a, -17), and one for both, ions and water (claudin-2). Interestingly, some of the barrier-forming claudins form the barrier in a charge-selective manner too (e.g. claudin-4, -8, -14). Since the crystal structure of claudin-15 has been resolved it is a major topic of several labs to elucidate the structural design of a complete claudin-based channel. It is unambiguous that this channel is formed by extracellular loops originating from two or more claudin protomers arranged in cis and/or trans position. Recent data from our lab are based on structure-function studies investigating detailed channel or barrier properties. The proteins analyzed include claudin-2, -3, -5, -10a, -10b, and -17. Results comprise (i) structural features of the barrier-forming claudins 3 and 5, (ii) oligomerization properties of claudin-3, -10a and -10b, (iii) description of the pore formed by claudin-2 being common for cations and water, and (iv) structural features of claudin-17 protomers capable of anion channel formation.

(COI: No)

S29-5

Molecular mechanisms of claudin function and regulation: Implications for physiology, pathobiology, and therapy

Turner, Jerrold (*Univ. of Chicago, Chicago, USA*)

Intercellular tight junctions form paracellular seals that are selectively permeable. To define the mechanisms of paracellular permeability, we developed a trans-tight junction patch clamp approach and showed that individual pores are actively gated similar to transmembrane ion channels. To define regulation of these pores, we explored the molecular basis by which casein kinase-2 (CK2) inhibition reduces paracellular cation flux. CK2 inhibition increased the mobile pool of claudin-2 at the tight junction by triggering assembly of tripartite complexes that also included ZO-1 and occludin, thereby preventing claudin-2 from forming paracellular pores. Dephosphorylation of the CK2 target serine 408 within the occludin cytoplasmic tail regulates assembly of these tripartite complexes. Consistent with this, CK2 inhibition reversed IL-13-induced, claudin-2-dependent increases in intestinal epithelial paracellular cation permeability, both in vitro and in vivo. To determine the potential relevance of increased claudin-2 expression in inflammatory bowel disease (IBD), experimental IBD was induced in claudin-2-deficient mice and CK2 inhibitor-treated mice; both were markedly protected from disease. In contrast, CK2 inhibitor treatment had no effect on claudin-2-deficient mice, indicating that the benefit provided by CK2 inhibition was claudin2-dependent. As a whole, these data provide novel insight into molecular mechanisms of claudin-2 pore function, regulation, and impact in disease and suggest that exploitation of these processes, e.g. by CK2 inhibition, may be beneficial in human disease.

(COI: No)

S29-6

Recent advances in claudin binder platforms and their contribution to drug development

Kondoh, Masuo; Yagi, Kiyohito (*Grad Sch Pharm Sci, Osaka University*)

Claudins (CLDNs), a tetra-transmembrane protein family with 27 members, are components of tight junction-seals. They prevent the free movement of solutes across epithelial cell sheets. CLDNs are frequently overexpressed in malignant tumors and are co-receptors for hepatitis C virus (HCV). Thus, they are promising targets for drug development. A popular strategy for drug development against membrane proteins is the preparation of binders to their extracellular region. However, recombinant CLDN proteins are difficult to prepare, which slowed the proof-of-concept for CLDN-targeted drug development. Several studies have provided insights into CLDNs as targets for drug development. C-terminal fragment of *Clostridium perfringens* enterotoxin (C-CPE) was identified as the first CLDN binder, proving that CLDNs can be targets for enhancing mucosal drug absorption, treating cancer, and mucosal vaccination. C-CPE binding to CLDN-3, -4, -6, and -9 has low CLDN-specificity; CLDN-specific binding is a requirement for clinical application. Therefore, anti-CLDN monoclonal antibodies have been developed as CLDN binders; some of these have anti-tumor activity. Anti-CLDN-1 antibodies prevented *in vitro* and *in vivo* HCV infection without apparent adverse effects. Moreover, the first three-dimensional structure of CLDN was determined this April, which will greatly facilitate future CLDN-targeted drug development. Here, we present an overview of CLDN-targeted drug development from the perspective of advances in platforms to create CLDN binders. The authors have no conflicts of interest.

(COI: No)

Symposium 30

Contents and view points necessary for the co-medical education of anatomy and physiology

(March 22, 9:00～10:30, Room D)

S30-1

What is the goal of physiology in education for allied health professionals?

Watanabe, Masaru (*Grad Sch Front Health Sci, Tokyo Met Univ, Tokyo, Japan*)

There are many problems in physiology education in training courses for allied health professionals. First, the students who enter the courses are unequal in knowledge level of basic sciences. However, in most cases, the students have to study physiology in the first school year of the courses. Second, basic medicine education occupies only limited hours in the courses, although knowledge of medical sciences what the students have to know is expanding. Third, in many cases, there are only one or two teaching staffs of physiology in the courses. Finally, in some cases, questions about physiology in a state examination for the license to allied health professionals are not prepared by physiologists. In this talk, the author will give some suggestions what is the goal of physiology in education for allied health professionals under such a limited conditions.

(COI: No)

S30-2

What kind of anatomy education is required for pharmacy students?

Suzaki, Etsuko (*Sch.Pharm.Shujitsu Univ., Okayama, Japan*)

In co-medical fields such as nursing, physical therapy, and clinical radiology, there has been an increasing request for practical training in the education of human anatomy. In Hiroshima University School of Medicine, for example, almost 2,000 co-medical students from 29 different schools experienced dissection practices last year. Among these students, only about 40 students were from the pharmacy department, and the interest to human anatomy can hardly be said to be high in the pharmaceutical field. Eight years have passed since pharmacy department introduced the six-year system of education, in which pharmacists who can play an active role as a member of the team medical care are intended to be trained. Thus, the pharmacy students will be required to learn basic medicine such as anatomy and physiology as well as other co-medical students learn. Needless to say, they learn the working mechanisms of medicines that have specific target organs or cells in the human body. Therefore, it is naturally important and essential for them to understand the structure, function and regulation mechanisms of the body. Through anatomical and histological practices, pharmacy students are able to acquire deeper understanding and more useful knowledge about the human body. Such experiences will help them to become the pharmacist who can cope equally as a member of the team medical care. In School of Pharmacy, Shujitsu University, anatomical and histological practices including human dissection have been tried. What practices are being introduced and what students have learned will be explained and discussed.

(COI: No)

S30-3

Teaching anatomy and physiology in nursing education

Nakatani, Toshio (*Div.Nurs.Fac.HealthSci.Kanazawa Univ., Kanazawa, Japan*)

I here describe how I teach anatomy and physiology to nursing students at the Department of Nursing, School of Health Sciences, Kanazawa University. Anatomy and physiology course names are Basic Anatomy, Basic Physiology and Human Physiology. The textbook for them is Anatomy and Physiology, one of a series of systematic nursing lecture courses, Igakushoin. They are taught to 1st year nursing students. Basic Anatomy and Basic Physiology are each taught via one 90-minute class per week, with 15 lectures and 1 examination in the first semester, giving two credits. Human Physiology is taught via 7 lectures and 1 examination in the second semester, giving one credit. Regarding macroscopic anatomical practice, students who want to examine a dissected corpse go to the anatomical dissection room two times, in the morning on a Saturday in June, where they can observe the inner organs, muscles, blood vessels and nerves. Osteological practice is performed in one of the 15 lectures, during which the students observe the bones. I do not teach microscopic anatomical practice. I lecture according to the above textbook. I also distribute copies of papers and other books in combination with the textbook, as well as a collection of questions for self-teaching. I use Powerpoint with a computer touch panel, Windows 8 or a document presentation device in my lectures because I write various things while I am lecturing. I check attendance using a roll card, on which the students can also evaluate my lecture. The examination questions are true-false problems or multiple-choice questions.

(COI: No)

S30-4

Anatomy education for undergraduate health professionals needs improvement, particularly anatomy practice

Kawamata, Seiichi (*Inst.Biomed.HealthSci., Hiroshima Univ., Hiroshima, Japan*)

Anatomy education for undergraduate health professionals should principally deal with all parts of the body at macro- and microscopic levels and an appropriately long time should be allocated to classes and practice. The most important fields of anatomy differ among the great variety of courses for health professionals. Nursing students are mainly interested in the thoracic and abdominal organs, whereas physical therapy (PT) and occupational therapy (OT) students have to understand the musculoskeletal system. Thus, anatomy education should be customized for students depending on their specialty.Practice is very important. All students on health care professional courses should observe dissected human cadavers at least once in order to obtain a better understanding of human structures and to correct prejudices and avoid possible misunderstandings. Nursing, PT and OT students should observe the brain. Exposure to dissected cadavers provides a good opportunity for students to learn about individual variability, to think about life and death, and to nurture their professionalism. Microscopic observation is very useful to understand functions of cells and organs. Furthermore, PT and OT students should dissect the musculoskeletal system by themselves. However, anatomy practice differs considerably in terms of method, duration and quality in Japan, even within the same course. A considerable proportion of undergraduate health professionals have no chance to observe or dissect cadaveric materials. It is thus important to improve anatomy education, especially practice, for undergraduate health professionals.

(COI: No)

Symposium 31

Imaging studies of memory processes with various animal models

(March 22, 9:00～10:30, Room F)

S31-1

Learning-induced Changes of Neural and Behavioral Responses to Chemosensory Stimuli in *C. elegans*

Iino, Yuichi[1]; Kunitomo, Hirofumi[1]; Ohno, Hayao[1]; Sato, Hirofumi[1]; Satoh, Yohsuke[1]; Iwata, Ryo[1,2] (*[1]Dept Biol Sci, Grad Sch Sci, Tokyo Univ, Tokyo, Japan; [2]Present Addr, RIKEN Ctr Dev Biol, Kobe, Japan*)

The nematode *C. elegans* is an excellent model organism for elucidating the neural basis of behavioral plasticity because of its simple nervous system and genetic manipulability. It senses various chemicals, processes the sensory information and shows behavioral output called chemotaxis. The direction of chemotaxis changes depending on previous experience. For example, after cultivation at a high concentration of salt, it migrates to high concentration of salt, while it will avoid high concentration of salt after cultivation at a low concentration of salt. These observations suggested that worms form a memory of salt concentration and recognize the difference between current concentration and previous salt concentration to determine the direction of movement. Neural activity imaging suggested that transmission of information from the sensory neuron to the first-layer interneurons is the major site of neural plasticity. Mutants of the phospholipase C/diacylglycerol/protein kinase C pathway migrated to abnormally higher or lower salt concentrations, suggesting that the activity of this pathway, which acts in the sensory neuron, biases the movement towards higher salt concentrations. Observations using a FRET reporter indicated that diacylglycerol level in the sensory neuron changes by the change in sensory input, suggesting that the diacylglycerol pathway is involved in either formation or read-out of the concentration memory.

(COI: No)

S31-2

Hippocampal neural circuit dynamics imaged during spatial behavior in mice

Sato, Masaaki[1,2] (*[1]PRESTO, Japan Science and Technology Agency, Kawaguchi, Japan; [2]RIKEN Brain Science Institute, Wako, Japan*)

In 1940s, the Canadian psychologist Donald Hebb proposed the theory of "cell assembly", in which he postulated neurons acting together are arranged into groups to form the brain basis of mental representation. Our research aims to elucidate the principle of how such functional circuits emerge, operate and change during repeated experience and learning. To directly visualize the dynamics of neuronal circuit activity in awake behaving animals, we have developed a set of new technologies, such as transgenic mice that express new fluorescent calcium indicator proteins in the brain, a virtual reality (VR) set-up for head-fixed mice and automated image analysis software. Mice head-fixed above an air-supported spherical treadmill were allowed to run freely in a computer-generated VR environment rendered on a wide LCD monitor that provided visual feedback in response to running. The mice trained in a virtual linear track task learned to exhibit spontaneous alteration of running and standing still, which allowed us to study behavioral-state dependent changes of hippocampal neuronal ensemble dynamics with two-photon calcium imaging. To test whether mice can discriminate a particular place in VR, we have recently established a new hippocampus-dependent virtual spatial recognition task. Our ongoing imaging experiments in mice performing this task will provide important insights into hippocampal neural network dynamics underlying memory-guided spatial behavior.

(COI: No)

S31-3

Visualization of Neural Representations of Memory

Matsuo, Naoki[1,2] (*[1]Dept Mol Behav Neurosci, Grad Sch Med, Osaka Univ; [2]PRESTO, JST, Saitama, Japan*)

Memories are presumably stored in a specific small subset group of neurons sparsely distributed in the brain in response to various sensory experiences. One of the major difficulties in studying the mechanism of cognitive functions including learning and memory is the identification of the "functional" neuronal populations among the hundred billions of neurons in mammalian brain. We have developed a transgenic system in mice that allows us a genetic manipulation in those neurons activated by a given behavioral stimulus during a limited time window. The mice express tetracycline-regulated transactivator (tTA) under the control of promoter of the c-fos gene, one of the immediate-early genes whose expression is rapidly and transiently induced in response to neuronal activities. The transgenic system provides a distinctive tool for visualizing the dynamism of neuronal ensembles representing a given information or memory.

(COI: No)

S31-4

Chemical tools to control cellular chemistry

Furuta, Toshiaki (*Dept Biomol Sci, Faculty of Sci, Toho Univ, Chiba, Japan*)

Caged compounds are designed synthetic molecules so that their original biological activities are temporally masked by covalently attached photo-caging groups. After being applied into live cells or tissue samples, appropriately designed caged compounds can manipulate various cellular processes such as neuronal signaling, intracellular signal transduction and gene expression upon photo-irradiation. The purpose of our study is to develop new photo-responsive chemical tools for controlling and monitoring cellular processes with high spatial and temporal resolution. We designed and synthesized brominated hydroxycoumarin (Bhc) chromophores as photo-responsible protecting groups which can be activated under one and two-photon excitation conditions with improved photochemical efficiency. The protecting groups have been applied to making caged compounds of low molecular weight organic compounds including neurotransmitters and second messengers. Thus, we demonstrated photo-manipulation of sperm motility using Bhc-caged cyclic nucleotides. Intracellular signaling in immature T cells can be controlled with subcellular spatial resolution using Bhc-caged DAG and flashes of focused UV light. Modular approaches to preparing caged compounds of DNAs and RNAs enabled photo-mediated gene expression in mammalian cells. One barrier to using conventional caged compounds in in vivo applications is the lack of cell type specificity because the compounds are not genetically encoded. To overcome the problems, we have developed new modular Bhc-caged compounds which can be photo-activated with cell type specificity.

(COI: No)

S31-5

Morphological analysis of memory circuits in rat, rabbit, and marmoset brains

Honda, Yoshiko (*Dept.Anat.Sch.Med.Tokyo Women's Med.Univ., Tokyo, Japan*)

The development of various techniques for visualizing memory circuits has recently enabled us to connect morphological information to functional information more easily. Research using various genetically modified animals is also increasing, and it is now essential to gain a sufficient understanding of the morphological information, i.e., the normal pattern of neuronal connections in each animal species, in advance of functional analyses of the various model animals. Here, we introduce some of the morphological features of memory circuits in rat, rabbit, and marmoset brains that have been clarified to date. The most essential portion of a memory circuit can be predicted to be generally preserved over rodents to primates; to elucidate such fundamental connections, we investigated neuronal connections between the hippocampus and parahippocampal cortices (i.e., the presubiculum, parasubiculum, and entorhinal cortex), in each species. Standard tracers, such as HRP, CTB, and BDA, were injected into the hippocampal body or several parahippocampal cortices, enabling input-and-output connections between each area on the cell mass level to be investigated. In addition, the palGFP-expressing Sindbis virus vector was used to analyze axonal arborization and termination of single neurons, particularly in the rat.

(COI: No)

Symposium 32

Chrono-network ~Molecular Physiology/ Anatomy Cross-talking with Biological Time

(March 22, 9:00～10:30, Room G)

S32-1

The chrononetwork and hypoxia signaling pathways

Ikeda, Masaaki[1,2]; Kumagai, Megumi[1,2]; Nakajima, Yoshihiro[3]; Ueno, Munehisa[4]; Okabe, Takashi[4] (*[1]Dept Physiol, Sch Med, Saitama Medical Univ, Moroyama, Japan; [2]Mol Clock Project, Res Center for Genomic Med, Saitama Medical Univ, Hidaka, Japan; [3]Biofunctional Regulation Res Group, Health Res Inst, AIST, Takamatsu, Japan; [4]Dept Uro-Oncol, Saitama Medical Univ, Hidaka, Japan*)

Hypoxia sensing is an important homeostatic mechanism in mammals. Hypoxia-inducible factors (HIFs), which belong to the basic helix-loop-helix-Per-Arnt-Sim transcription factor family, function as sensors for hypoxia and are involved not only in the transactivation of hypoxia-induced genes but also in pathologic processes such as carcinogenesis and the progression and metastasis of many cancers. Recently, we demonstrated that HIF-1α activated transcription of the *Period2* (*Per2*) gene via an HIF response element and enhanced the amplitude of the *Per2* promoter rhythm in cells. HIFs and PER2 play important roles in cancer progression. We examined PER2 expression by Western blotting in renal cancer cell lines; however, almost no expression was detected. The protein level of HIF-1α was also negligible in these cells. Real-time monitoring of *Per2* promoter activity using a destabilized luciferase reporter fused with the *Per2* promoter revealed that the oscillation of *Per2* promoter activity was highly diminished in all of the renal cancer cell lines examined. Thus, the expression levels of PER2 and HIF-1α are determining factors in the circadian oscillation of clock genes in cancer cells.

(COI: No)

S32-2

Molecular signals connecting Chrono-network of the adaptation systems

Tamaru, Teruya (*Dept. of Physiol., Toho Univ. Sch. of Med.*)

Among various adaptation systems against environments/ stresses/ aging, circadian (clock) system has daily-periodicity and synchronizing properties to external stimuli. Circadian system is driven by cell-autonomous molecular clocks (core circadian oscillator) consisted with clock genes/ proteins; Bmal1, Clock, Cry, Per. Dysfunctional clocks become risk factor for various diseases, likely via down-regulated adaptation (protection, repair, etc.). Thus, manipulating (chrono-) network of the adaptation systems via circadian signal would become potential medical strategy. So far, we found; 1) protein modification-interaction oscillator controlling core circadian oscillator (Science 2005, Nature 2007, Nat Struct Mol Biol 2009), 2) cell injury stresses -evoked clock synchronization and activation of adaptation pathways (PLoS ONE 2011, 2013). Here, as molecular signals connecting chrono-network of the adaptation system, we will discuss about BMAL1, CRY, CK2, HSF1, etc., as the players in the chrono-networking among the core circadian oscillator, protein modification-interaction oscillator and adaptation systems

(COI: No)

S32-3

Direct interaction between tumor suppressors and the circadian rhythm

Miki, Takao (*Dept Mol Biol, Grad Sch Med, Kyoto Univ, Kyoto, Japan*)

Accumulating epidemiological evidence suggests that cancer and the circadian clock have close interplay, although direct molecular evidence in mammalian systems remains scarce. The circadian clock is the daily oscillation of biological processes and believed to have close interplay with many fundamental cellular pathways including metabolism. Previous studies have shown that mice lacking Period 2 (Per2), a critical component of circadian clock, was cancer prone, suggesting that clock genes could be involved in tumor suppression and hence, PER2 and other tumor suppressors may function in an integrated network. We screened several tumor suppressors for their possible clock functions and identified two major tumor suppressors, p53 and PML. We also found that p53 and PML exert their clock functions by regulating Per2. These studies highlight the direct connection between tumor suppression and the circadian clock. Since the circadian clock is known to regulate cellular metabolism, some of the metabolic changes found in cancer cells may also be explained by loss of tumor suppressors involved in circadian clock regulation.

(COI: No)

S32-4

A chemical biology approach to dissect chrononetwork

Hirota, Tsuyoshi (*ITbM, Nagoya Univ, Nagoya, Japan*)

The circadian clock is an intrinsic time-keeping mechanism that coordinates the daily rhythms of numerous physiological processes, such as sleep/wake behavior, hormone secretion, and metabolism. Circadian rhythms are generated in a cell-autonomous manner through transcriptional regulatory networks of the clock genes. To develop a new approach for the circadian clock research, we applied chemical biology that uses chemicals to investigate biological machinery. We have established a cell-based high-throughput circadian assay and conducted phenotype-based chemical screens. From hundreds of thousands of small molecules, we identified two compounds named longdaysin and LH846 that potently lengthen the period of the circadian clock through inhibition of casein kinase I family proteins. More recently, we discovered a new class of period lengthening compound named KL001 that specifically interacts with the core clock protein CRY to inhibit its degradation. We demonstrated that KL001 inhibits glucagon-dependent induction of gluconeogenesis in mouse primary hepatocytes, based on a regulatory role of CRY in the pathway. KL001 is the first compound specifically targeting CRY and may provide an opportunity to enable clock-based control of gluconeogenesis. Furthermore, quantitative manipulation using compounds in combination with mathematical modeling enabled systems level understanding of the clock oscillation. These studies indicate effectiveness of chemical biology approaches for a better understanding of chrononetwork.

(COI: No)

S32-5

Cytosolic calcium rhythms in circadian pacemaker neurons: Current issues and future perspective

Ikeda, Masayuki (*Grad Schl Sci Eng, Univ Toyama, Toyama, Japan*)

Since presence of tetrodotoxin-resistant circadian Ca^{2+} rhythms (CCR) in suprachiasmatic nucleus (SCN) neurons was reported (Ikeda et al, *Neuron* 2003), numerous of newer findings progress the understanding of ionic rhythms in circadian pacemakers. I will overview these and discuss about future perspective in this filed. Cytosolic free Ca^{2+} is a ubiquitous signaling messenger, with plant cells showing strong CCR. However, corresponding CCR have not been reported in animal cells, other than in mature SCN neurons. For example, endocrine circadian oscillators in pupae of *Drosophila melanogaster* failed to display dynamic CCR (Morioka et al, *Nat Commun* 2012). In addition, we analyzed a SCN progenitor cell line that stably expresses YC3.6 (SCN2.2YC), but failed to observe CCR in these cells (Takeuchi et al, *Sci Rep* 2014). Because SCN2.2YC displays Per1-luciferase oscillations and expresses voltage-sensitive Ca^{2+} channels, other unveiled component(s) that are present in mature SCN neurons may be essential for the generation of CCR. It should be emphasized that machineries specific for pacemaker neurons may be present as we demonstrated that differential involvement of the C-terminal motif of clock gene Bmal1 between SCN neurons and fibroblasts, and that nuclear translocation of BMAL1 is more strictly regulated in SCN neurons (Ikeda and Ikeda, *J Neurosci* 2014). Candidate molecules and/or machineries linking molecular oscillations to ionic rhythms will be argued by comparing rhythms in lateral neurons, which are central pacemaker neurons in *Drosophila melanogaster*.

(COI: No)

Symposium 33

Frontiers in morphological and functional studies of neocortical circuits

(March 22, 9:00～10:30, Room H)

S33-1

Compartmental organization of synaptic inputs to parvalbumin-expressing inhibitory neurons in mouse neocortex

Hioki, Hiroyuki (*Morphol. Brain Sci., Grad. Sch. Med., Kyoto Univ., Kyoto, Japan*)

Neocortical GABAergic neurons are divided into at least three distinct subgroups by chemical markers: 1) parvalbumin (PV); 2) somatostatin (SOM); 3) other markers such as vasoactive intestinal polypeptide (VIP). PV neurons are a major component of neocortical GABAergic neurons, and considered to play a key role in higher-order brain functions and psychiatric disorders.

We have recently succeeded in visualizing dendrites and cell bodies of PV neurons completely by generating transgenic mice. Using the mice, we first analyzed excitatory and inhibitory inputs to PV neurons in the primary somatosensory cortex. Corticocortical glutamatergic inputs were more frequently found on the distal dendrites than on the soma, whereas thalamocortical inputs did not differ between the proximal and distal portions. GABAergic terminals were more densely distributed on the cell bodies than on the dendrites.

We further investigated which types of neocortical GABAergic neurons preferred the cell bodies of PV neurons to the dendrites. We revealed that the dendritic compartment principally received GABAergic inputs from PV neurons, while the somatic compartment received inputs from VIP neurons. This compartmental organization of synaptic inputs suggests that PV neurons communicate with each other mainly via the dendrites, and that their activity is effectively controlled by the somatic inputs of VIP neurons. In addition, this further suggests that PV neurons located in the superficial and deep cortical layers are simultaneously inhibited by vertically running VIP axons.

(COI: No)

S33-2

Dynamic behavior of inhibitory synapse on pyramidal cell

Kubota, Yoshiyuki[1,2,3] ([1]*National Institute for Physiological Sciences;* [2]*SOKENDAI, Okazaki, Japan;* [3]*JST-CREST, Tokyo, Japan*)

While the adult brain has long been considered hard-wired, recent in vivo imaging studies using excitatory or inhibitory synaptic markers have revealed a capacity for remodeling of neuronal connections. Here, we simultaneously monitor in vivo inhibitory synapse and dendritic spine dynamics across the dendritic arbor of pyramidal neurons in the adult mouse cortex using large-volume, high-resolution dual-color two-photon microscopy. We chronically monitored postsynaptic markers onto Layer 2/3 pyramidal neurons in the mouse primary visual cortex in vivo. We found that inhibitory synapses on dendritic spines are exceptionally dynamic as compared to other synaptic populations, due to the fact that a large proportion of them disappear and recur again in the same location on a timescale of days. In contrast to these inhibitory synapses on dually innervated spines, excitatory synapses on the same spines, as well as the spines themselves were extremely stable. Electron microscopic observation revealed that when the postsynaptic element of recurrent synapses observed in vivo disappeared, a presynaptic inhibitory axon could be found adjacent to the site of the recently removed inhibitory postsynaptic structure. Inhibitory synapses are often found on dendritic spines which receive direct thalamic input. Thus, the function of inhibitory synaptic remodeling at this locale may serve as a mechanism for gating feedforward excitation, rather than, as proposed for excitatory synapses, as a mechanism to remodel circuitry and exchange partners.

(COI: No)

S33-3

Macroscopic functional organization of natural visual representation in the human cortex

Nishimoto, Shinji[1,2] ([1]*Center for Information and Neural Networks, National Institute of Information and Communications Technology, Osaka, Japan;* [2]*Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan*)

One of the long-term goals of systems neuroscience is to reveal the functional and anatomical principles underlying our natural perception and behavior. Recently, we developed a quantitative framework to understand the natural visual representation in the human visual cortex via modeling movie-evoked brain activity measured using fMRI (functional magnetic resonance imaging). The framework aimed to reveal feature spaces of visual representation within cortical areas of interest as well as elucidate how the representation was distributed across the cortex. Using this framework we recovered the representations of spatiotemporal and semantic information in the human visual cortex as well as their macroscopic functional structure. The framework is general and can also be used to decode visual experiences and assess quantitative differences in cortical representation between different cognitive states or individuals. Our framework is a powerful tool to facilitate the quantitative understanding of functional structures in the human cortex under natural conditions.

(COI: No)

S33-4

Visual object recognition based on short-term memory in mice

Shibuki, Katsuei (*Dept Neurophysiol, Brain Res Inst, Niigata Univ, Niigata, Japan*)

Mice are important experimental animals, since many new techniques are available in the experiments using mice. We found that mice have visual short-term memory of objects. In a memory-dependent task, a cue object was presented at center of the display in front of mice, and two choice objects including the original cue object were presented with a delay interval of 20 s. Mice could select the original cue object based on short-term memory. After this visual short-term memory sessions were finished, we confirmed that mice could similarly differentiate a pair of alphabets, which they had never seen before, based on short-term memory. These results indicate that mice can recognize and memorize visual objects as complex as alphabets. Higher visual areas responsible for object recognition are unknown in mice. To identify the responsible areas, we used an association memory paradigm. After mice were exposed a combination of an object and a sound, they could choose the object based on the associated sound cue only. We further investigated cortical responses to the sound cue using flavoprotein fluorescence imaging. The cortical responses to the sound cue were observed in the auditory cortex and higher visual areas located dorsally to the auditory cortex, suggesting that the activated higher visual areas may play an important role in objects recognition. As expected, we found object-specific neuronal activity in these areas using two-photon microscopy. Interestingly, the short-term memory, associative memory, and the memory based-cortical activities were not found in mice with reduced diversity of clustered protocadherin α.

(COI: No)

Symposium 34

Crosstalk between nervous and immune systems

(March 22, 9:00～10:30, Room I)

S34-1

Local Neural Activation Enhances Inflammation via Gateway Reflex

Murakami, Masaaki (*Inst. Genetic Medicine and Grad. Sch. Med., Hokkaido Univ., Sapporo, Japan*)

The CNS is an immune-privileged environment that can be compromised by an accumulation of immune cells, particularly pathogenic T cells. Using a transfer system from the multiple sclerosis model, EAE, we show autoreactive Th17 cells accumulate in the CNS via dorsal blood vessels in the 5th lumbar-cord (L5 cord). These vessels excessively express various chemokines including CCL20, which attracts the autoreactive Th17 cells, in a manner dependent on regional nerve activation mediated by anti-gravity responses on the soleus muscle, which enhances the NFkB signal in the L5 vessels. This activation increased vessel blood flow in the L5 cord via the activation of L5 sympathetic neurons. On the other hand, the inhibition of norepinephrine signaling in vivo suppressed CCL20 expression, pathogenic T cell accumulation in the L5 cord, and EAE-development. Consistent with these observations, norepinephrine enhanced a synergistic NFkB signal after IL-17A and IL-6 stimulation. We term these neuroinflammatory relationship as Gateway Reflex, which describes that neural activation can be transformed into an inflammatory-signal in the dorsal vessels of the L5 potentially leading to autoimmune diseases like EAE. Thus, the Gateway Reflex, may offer new therapeutic targets for various diseases and disorders particularly in the CNS.

(COI: No)

S34-2

RGMa modulates T cell responses and is involved in Th17 cell-induced neurodegeneration in autoimmune encephalomyelitis

Yamashita, Toshihide (*Grad.Sch.Med.Osaka Univ., Osaka, Japan*)

Multiple sclerosis (MS) is an autoimmune disease caused by myelin-specific T cells that induce an immune response against the brain and spinal cord. We demonstrated that repulsive guidance molecule-a (RGMa) is a promising new target for the treatment of MS. RGM was originally identified as a membrane-bound protein with repulsive and growth cone collapse-inducing activities in the chick retinotectal system. Expression analysis revealed that RGMa is expressed in bone marrow-derived dendritic cells (BMDCs) and that CD4+ T cells express receptor for RGMa. Treatment with neutralizing antibodies to RGMa prevented mouse experimental autoimmune encephalomyelitis (EAE) and reduced invasion by inflammatory cells. In humans, RGMa-specific antibody could modulate T cell proliferative responses and cytokine expression in peripheral blood mononuclear cells isolated from patients with relapsing-remitting MS. These results show that RGMa-specific antibody suppresses T cell response to antigens. Furthermore, we recently demonstrated that RGMa is associated with neurodegeneration in EAE. RGMa was highly expressed in interleukin-17-producing CD4+ T cells (Th17 cells). We induced EAE by adoptive transfer of myelin oligodendrocyte glycoprotein (MOG)-specific Th17 cells. Inhibition of RGMa improved EAE scores and reduced neuronal degeneration without altering immune or glial responses. Th17 cells induced cultured cortical neuron death through RGMa-neogenin and Akt dephosphorylation. Our results demonstrate that RGMa is involved in Th17 cell-mediated neurodegeneration.

(COI: No)

S34-3

Mechanisms of brain inflammation after stroke

Yoshimura, Akihiko; Ito, Minako; Shichita, Takashi (*Dept Microbiol & Immunol., Keio Univ. School of Med., Tokyo, Japan*)

It has been well established that the interleukin-23 (IL-23)-IL-17 axis plays essential role in experimental autoimmune encephalomyelitis (EAE) which is a well-established Th17 cell-mediated brain and spinal cord inflammation. Stroke or brain ischemia is one of the major causes of death and disability worldwide. Post-ischemic inflammation is an essential step in the progression of brain ischemia-reperfusion injury. In a mouse stroke model, we have reported that IL-23 and IL-17 play essential roles in infarct volume growth in the brain ischemia model. IL-23 is produced from infiltrating macrophages, which induces IL-17 from T cells. IL-17 is mainly produced from $\gamma\delta$ T cells and promotes delayed (day 3-4) ischemic brain damage. Furthermore, we demonstrate that peroxiredoxin (Prx) family proteins released extracellularly from necrotic brain cells induce expression of inflammatory cytokines including interleukin-23 in macrophages through activation of Toll-like receptor 2 (TLR2) and TLR4, even though intracellular Prxs have been shown to be neuroprotective. Extracellular Prxs are cleared by macrophages, which is important for the resolution of brain inflammation. We have clarified a scheme of brain inflammation after stroke.

(COI: No)

S34-4

Translational Research in Neuroimmunological Disorders

Yamamura, Takashi (*Dept Immunol, Nat Inst Neurosc, NCNP, Kodaira*)

Pathogenesis of neurological disorders was traditionally investigated by means of clinical, pathological, and electrophysiological techniques. More recently, genetics and radiology have contributed greatly to better diagnosis and classifications of rare diseases. However, immunological approaches in the past decade have been most successful in the development of drugs. In fact, interferon-β, anti-Vα4 integrin antibodies, and fingolimod have been developed for multiple sclerosis (MS), an autoimmune disease, accompanying inflammatory demyelinating lesions in the central nervous system (CNS). Of note is that these emerging therapies gained seeds from the academic research, indicating the importance of translational research. In this symposium, I will talk about two ongoing translational studies that we designed. The first one, application of anti-IL-6 receptor antibody therapy for neuromyelitis optica (NMO), was initiated following the discovery that IL-6 dependent plasmablasts producing autoantibodies are increased in the peripheral blood of patients with NMO (PNAS 2011). Another study, development of NKT cell ligand therapy for MS, aims at obtaining proof of concept to ask if the efficacy of glycolipid OCH is efficacious not only in mouse MS model (Nature 2001) but also in human. Unexpected results from these studies will be highlighted with regard to the scientific merit and novelty.

(COI: No)

S34-5

Gut immunity and neuroinflammation

Miyake, Sachiko (*Juntendo Univ. Sch Med., Tokyo Japan*)

Multiple sclerosis (MS) is a chronic autoimmune disease targeting the central nervous system (CNS). Recent increase in the number of MS patients in Japan is probably attributed to the environmental changes rather than genetic changes. The intestine has lately received much attention as a potential location for the regulation of immune cells. We and other groups previously showed that alterations of gut environment could lead to the amelioration of experimental autoimmune encephalomyelitis (EAE), a rodent model for MS. We investigated the characteristics of myelin reactive T cells in the gut and the molecular mechanism of the way they could influence on CNS autoimmunity using myelin oligodendrocyte glycoprotein (MOG) reactive T-cell receptor transgenic (2D2) mice. Adoptively transferred 2D2-CD4+ cells among intraepithelial lymphocytes (IELs) ameliorated EAE. The transferred IEL were found to migrate into the CNS and up-regulated several immune regulatory molecules. 2D2-CD4+ IELs exhibited the suppressive activities on T cell proliferation. These findings suggested that gut is an important place to regulate the function of autoreactive T cells and neuroinflammation.

(COI: No)

Symposium 35

Neuronal mechanisms of respiratory control in the medulla and spinal cord: integrative view of the anatomy and function

(March 22, 9:00〜10:30, Room J)

S35-1

Recent progress in understanding of a respiratory rhythm generation center, pFRG

Ikeda, Keiko[1]; Onimaru, Hiroshi[2] (*[1]Biology, Hyogo Col. Med., Hyogo, Japan; [2]Dept Physiol, Showa Univ Sch Med, Tokyo, Japan*)

The pivotal role of the respiratory center in homeostasis is to control ventilation to maintain optimum pCO_2/pH and pO_2 in extracellular fluids. Such information is perceived by peripheral and central chemoreceptors. As regarding the central chemoreception, the understanding of the cytoarchitecture of respiratory control center in the brainstem has been progressed greatly in the last two decades. One of the milestones was the discovery of *PHOX2B* mutations in patients of congenital hypoventilation syndrome which shows abrogation or a great reduction of the sensitivity to hypercapnia. Another was the identification of unique expression pattern of the paired-type homeobox gene *Phox2b*. Others and we have reported the existence of a small population of Phox2b-expressing neurons in the parafacial respiratory group (pFRG)/the retrotrapezoid nucleus (RTN) in the brainstem. The preservation of CO_2 sensitivity even after blockade of Na^+ channels and Ca^{2+} channels in the Phox2b-positive pre-inspiratory (Pre-I) neurons in pFRG/RTN indicated that the Pre-I neurons indeed possess CO_2 sensor molecule(s) whose transcriptional expression may be directly regulated by Phox2b. To uncover the sensor molecules and to facilitate understanding of respiratory neural network, we have recently generated a bacterial artificial chromosome transgenic rat line harboring a fluorescent protein under the control of a mouse *Phox2b* promoter/emhancer. Here we show a new insight into cytoarchitecture together with electrophysiological function of the Phox2b-positive neurons using this transgenic rat.

(COI: No)

S35-2

Anatomy of the respiratory rhythmogenic kernel: pre-Bötzinger complex of the medulla

Okada, Yasumasa[1]; Yokota, Shigefumi[2] (*[1]Clin Res Ctr, Murayama Med Ctr, Tokyo, Japan; [2] Dept Anat Morphol Neurosci, Shimane Univ Sch Med, Izumo, Japan*)

The preBotzinger complex (preBotC) of the ventrolateral medulla is the kernel for respiratory rhythm generation. We analyzed anatomical connection of preBotC neurons to and from other respiratory related medullary regions in rats. In retrograde and anterograde tracing, Fluoro-gold (FG) and biotinylated dextran amines (BDA) were microinjected into the unilateral preBotC, respectively. Putative rhythmogenic neurons were identified in the preBotC by immunostaining of neurokinin-1 receptor (NK1R) and somatostatin (SST). A large number of FG-labeled neurons were distributed in the contralateral ventrolateral medulla throughout its rostrocaudal extent. One-third of FG-labeled neurons were immunoreactive for NK1R in the preBotC. In anterograde tracing, we found that BDA-labeled boutons were in contiguity with dendrites and somata of neurons that were double-labeled with NK1R and SST in the contralateral preBotC. When the preBotC region was electron microscopically examined, we found BDA-labeled axon terminals making synaptic contacts with somatic or dendritic profiles of NK1R-immunoreactive neurons in the contralateral preBotC, and most of the synapses observed were of an asymmetrical type. We elucidated the anatomical pathways (1) from the preBotC in one side to the contralateral preBotC, and (2) from the preBotC directly to the bilateral hypoglossal premotor and motor areas as well as to the nuclei tractus solitarius. These connectivities would be the anatomical basis for bilaterally synchronized respiratory rhythmogenis and robust control of breathing.

(COI: No)

S35-3

Physiology of the pre-Bötzinger Complex

Koshiya, Naohiro (*NIH-NINDS, Bethesda MD, USA*)

The ventrolateral medulla contains respiratory neurons. While many of them (the ventral respiratory group) project to spinal motor nuclei, a gap was found at a mesorostrocaudal level, where respiratory neurons were mostly propriobulbar. Discovered there was a bilaterally distributed population pacemaker for breathing rhythm, named pre-Botzinger complex (pBC). Within the heterogeneous reticular formation, some pBC inspiratory (pacemaker) neurons possess intrinsic rhythm generation mechanisms. They are capable to generate periodic bursts, even in isolated conditions, with balanced conductances of a persistent Na+ (gNaP) and K+ dominated leak (non-voltage-gated), arguably with other conductances (e.g., Ca2+). The pBC inspiratory pacemaker population is functionally connected together with mutual glutamatergic synapses, on each side of the brainstem and bilaterally by decussating axons, with which they synchronize their activities. Also within this positive recurrent recruitment system, other non-pacemaker (synaptic amplifier) inspiratory neurons conceivably contribute to the population-level generation of the central inspiratory drive. These pBC neurons express variety of synaptic and ligand receptors, with which the rhythmogenic kernel is functionally embedded in larger systems. Via such supersets' afferents, the pBC monitors environmental information, e.g., from the arterial and central chemoreceptors, as feedback; furthermore the pBC cells have chemosensitivity by themselves for a fundamental homeostasis. We will review the pBC's inspiratory motor rhythm generation mechanisms in a multiscale perspective from cellular biophysics to synaptic, microcircuit, population, and network levels.

(COI: No)

S35-4

Structure and function of respiratory neuronal circuits of the high cervical spinal cord

Oku, Yoshitaka[1]; Hayakawa, Tetsu[2] (*[1]Dept Physiol, Hyogo Col Med, Nishinomiya, Japan; [2]Dept Anat, Hyogo Col Med, Nishinomiya, Japan*)

Animals spinalized at the C1 level can generate respiratory rhythm (Aoki et al. 1980). However, since administration of curare abolishes phrenic activity, the respiratory rhythmicity is thought to be supported by feedback inputs from cutaneous and chest wall proprioceptors. In the current concept, eupnea, defined by the breathing pattern comprising inspiration, post-inspiration, and expiration, requires the integrity of the pontine-medullary respiratory network. Essential structures for eupneic breathing have been postulated to extend from the pons to the pre-Botzinger complex. Structures caudal to the obex are thought to be unnecessary for eupneic breathing. Optical imaging using voltage-sensitive dyes led to discoveries of novel respiratory regions: the parafacial respiratory group (Onimaru and Homma, 2003) and the high cervical respiratory group (HCRG) in the spinal cord (Oku et al., 2008). This latter discovery motivated a re-examination of the role of spinal cord in respiratory rhythmogenesis. Jones et al. (2012) recorded phrenic (PNA) and hypoglossal (HNA) nerve activity in the perfused brainstem preparation of rat. Transverse transections at the pyramidal decussation not only abolished PNA immediately, but also progressively deprived HNA amplitude and rhythm. Transverse transections at the first cervical spinal segment level did not abolish HNA rhythmicity. The result contradicts the current concept of the genesis of eupnea, and indicates the importance of structures at the medullo-spinal junction.

(COI: No)

S35-5

Respiratory activity in the thoracic spinal cord

Iizuka, Makito (*Dept Physiol, Showa Univ Sch Med, Tokyo, Japan*)

The inspiratory and expiratory motor outputs are larger in the intercostal muscles positioned at more rostral and caudal segments, respectively. Such rostro-caudal gradient is kept in the *in vitro* preparation from neonatal rat. It is well documented that there are many propriospinal respiratory neurons in the thoracic spinal cord and anatomical studies showed no evidences that the inspiratory bulbospinal neurons have systematic patterns of connections to different segments. Therefore, such gradients in part could be generated by the excitatory respiratory propriospinal neurons with similar distribution. To examine this hypothesis, the respiratory-related neuronal activities were optically recorded from thoracic segments in the brainstem-spinal cord preparations from neonatal rats stained with voltage-sensitive dye. Respiratory-related signals were detected from ventral surface of all spinal segments examined (T1-T13). The blockade of the synaptic transmission in the thoracic spinal cord by the low Ca^{2+} superfusate blocked all respiratory signals, suggesting that these signals should come from spinal neurons. Areas of the optical signals evoked by the antidromic activation of the motoneurons were restricted in the lateral areas where the respiratory signals were observed. Therefore, the medial areas would come from the spinal interneurons. In both areas, more rostral thoracic segments showed larger inspiratory-related signals. These results support our hypothesis.

(COI: No)

S35-6

Integrative view of respiratory control mechanisms in the pons, medulla and spinal cord

Onimaru, Hiroshi[1]; Koizumi, Hidehiko[2] (*[1]Dept Physiol, Showa Univ Sch Med, Tokyo, Japan; [2]USA*)

Central respiratory neuronal activity is primarily produced in the respiratory rhythm generator of the ventral medulla. The respiratory rhythm and motor patterns are regulated by various information from many regions in the lower brainstem including the pons while they are transmitted to motor neurons (e.g. in the spinal cord) via pre-motor neurons. The normal respiratory motor pattern consists of basically three or four phases; pre-inspiratory, inspiratory, post-inspiratory and late-expiratory. A number of different types of preparations from mainly mice or rats have been used for analyses of respiratory rhythm and pattern generation; medullary slice (newborn or juvenile), en bloc brainstem-spinal cord preparation (newborn), decerebrate and arterially perfused preparation (newborn or juvenile) and in vivo preparation (any ages). These experimental variations that show the different motor output pattern of respiratory activity caused some controversy in the field. Recent studies demonstrated the significant role of the pons in formation of the respiratory burst pattern. On the basis of our knowledge from recent studies, we provide an integrative view of respiratory control mechanisms involving the pons, medulla and spinal cord.

(COI: No)

Symposium 36

Frontier of functional and morphological research in epithelial tissues of digestive organs

(March 22, 16:00～17:30, Room C)

S36-1

Refined arrangement of monocarboxylate transporters (SMCT and MCT) in the intestine

Iwanaga, Toshihiko (*Grad. Sch. Med. Hokkaido Univ., Sapporo, Japan*)

Plant-derived dietary fibers and undigested carbohydrates are fermented by bacterial microflora in the large intestine, resulting in the productions of acetate, propionates, and butyrate, collectively called short-chain fatty acids (SCFAs). SCFAs are further classified into monocarboxylates together with lactate and ketone bodies. In order to absorb SCFAs as nutrients, the epithelium of the large intestine expresses a selective transporter, sodium-coupled monocarboxylate transporter (SMCT1). SMCT1 is localized only at the brush border of the large intestine. The gut epithelial cells also possess a proton-coupled transporter for SCFAs, MCT (monocarboxylate transporter)-1, on the basolateral membrane to transport SCFAs through the epithelium to internal milieu. Another type of SMCT2 with 59% identity with SMCT1 mediates the transport of monocarboxylates with low affinity as compared with SMCT1. Since its distribution was restricted to the jejunum and ileum, function of SMCT2 may be to absorb lactate from fermented milk and yoghurt and acetic acid rich in vinegar. The arrangement of high affinity SMCT1 and low affinity SMCT2 is biologically significant, as in the case of the kidney, where differential distribution of SMCT2 in the proximal urinary tubules and of SMCT1 in the distal part makes possible the effective re-absorption of lactate. MCT1 in the small intestine was expressed intensely in the basolateral membrane of crypt cells including dividing cells. Expression patterns of MCT1 in the skin, bone marrow as well as the small intestine suggest that monocarboxylates are favorite energy sources in self-renewing tissues.

(COI: No)

S36-2

The functional diversity of TJ-barrier in the digestive tract regulated by TJ-claudins in mice

Tamura, Atsushi; Tsukita, Sachiko (*Grad. Sch. of Front. Biosci. and Grad.Sch.of Med., Osaka Univ.*)

The properties of the tight junction (TJ) in digestive tracts are so different between organs/tissues. The permselective properties of TJ are thought to be mainly determined by the expression pattern of TJ component membranous protein claudins. What does different pattern of claudin based TJ permeability plays roles in multicellular organisms? This question has not been resolved well in many cases. In the liver, claudin-1, -2, -3 are dominantly expressed. Among them, claudin-1, -3 are barrier forming types of claudins and homogeneously expressed throughout the hepatic lobule while claudin-2 is channel forming type of claudin and predominantly expressed only around perivenous zone. Recently, we analyzed and found the bile flow of claudin-2 deficient mice were decreased by about a half compared to that of wild type mice because of the decreased expression of channel forming type of claudin-2 between sinusoidal flow and bile canaliculi flow. The bile canaliculi terminate in blind ends in the perivenous region and the bile flow starts from the perivenous region toward the periportal region. On the other hand, the sinusoidal blood streams start from the periportal region toward the perivenous region on the contrary to the bile flow. This counter current flow system seems adequatly maintained by the uneven distribution of cation/water permeable clauin-2. Thus, claudin based TJ function in the biological and pathophysiological system is discussed especially in claudin-2 deficient mice.

(COI: No)

S36-3

Molecular mechanism of morphofunctional regulation via PPARα autocrine modulation of Ca^{2+}-regulated exocytosis in mucous cells of gastric antrum

Tanaka, Saori[1]; Nakahari, Takashi[2] (*[1]Laboratory of Pharmacotherapy, Osaka University of Pharmaceutical Sciences, Takatsuki, Japan; [2]Department of Molecular Cell Physiology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan*)

In antral mucous cells, the Ca^{2+}-regulated exocytosis activated by ACh consists of an initial transient increase (initial phase) followed by a second slower decline (late phase). The ACh-stimulated exocytosis is modulated by PPAR α. However, we did not know how PPAR α modulates ACh-stimulated exocytosis. We studied the PPAR α actions in ACh-stimulated exocytosis of antral mucous cells. GW7647 (PPAR α agonist) enhanced the ACh-stimulated initial phase which was abolished by GW6471 (PPAR α antagonist). However, GW6471 produced a delayed, but transient increase (delayed increase) in the late phase. The inhibition of the initial phase and the delayed increase in the late phase were similarly induced by a PKG inhibitor or a NOS1 inhibitor. Moreover, in antral mucosae, ACh or GW7647 increased NO production and cGMP contents. On the other hand, Wortmannin (an inhibitor of PI3K) and Akt 2/2 kinase inhibitor (an inhibitor of Akt) also abolished the enhancement of initial phase and produced the delayed increase in the late phase during PPAR α activation in ACh-stimulated exocytosis. These observations suggest that AA/PPAR α autocrine mechanism stimulates NOS1 mediated via PI3K/Akt pathway leading to NO production and cGMP accumulation, which enhances the Ca^{2+}-regulated exocytosis in antral mucous cells.

(COI: No)

S36-4

Physiological effects of short-chain fatty acids on the intestinal epithelia - Difference between species and intestinal segments

Karaki, Shinichiro; Kuwahara, Atsukazu (*Lab Physiol, Sch Food Nutr Sci, Univ Shizuoka, Shizuoka, Japan*)

Short-chain fatty acids (SCFAs), 2-6 carbon monocarboxylic acids, are the predominant fermented products in the large intestine. They not only are absorbed as nutrients, but also stimulate intestinal mucosa inducing a variety of physiological responses including transepithelial ion transport. In the rat and guinea pig, it has been reported that SCFAs induce an anion secretion measured by the Ussing chamber technique. However, there had been no report that SCFAs induced a secretion in the human intestine until we reported the SCFA-induced anion secretion in the human terminal ileum at the Annual Meeting of the Physiological Society of Japan last year. We have found that SCFAs do not induce ion transport in any segments of the human colon, but induce anion secretion in the human terminal ileum. In the rat and guinea pig colon, it has been reported that propionate evokes anion secretion, but acetate does not. However in the human terminal ileum, the luminal addition of acetate also concentration-dependently evoked an anion secretion the same as propionate. Moreover, the luminal acetate-induced response was attenuated dependent on the concentration of propionate pretreated, and vice versa. These results suggest that the reflux of SCFAs from cecum to terminal ileum passing through the ileocecal valve may induce a fluid secretion in the human terminal ileum. These differences in the effects of SCFAS on intestinal epithelia between species and intestinal segments might be due to the food habit and/or the style of feces.

(COI: No)

S36-5

The molecular mechanism of intracellular Cl^- function in gastric cancer invasion and metastasis by regulating expression of cell adhesion molecules

Miyazaki, Hiroaki[1,2]; Marunaka, Yoshinori[1,2] (*[1]Dept Mol Cell Physiol, Grad Sch Med Sci, Kyoto Pref Univ Med, Kyoto, Japan; [2]Japan Inst Food Edu Health, Heian Jogakuin (St. Agnes) Univ, Kyoto, Japan*)

As tumors progress to increased malignancy, cells develop the ability to invade into surrounding normal tissues (the de-adhesion process) and through tissue boundaries to form new growths (the adhesion process) at sites distinct from the primary tumor; i.e., . It is generally accepted that the alterations of cell-cell and cell-matrix adhesion in metastatic tumor cells caused by changing their microenvironments may play critical roles in the metastatic process. Our recent studies show that the intracellular Cl^- plays important roles in fundamental cellular functions that would be involved in the cancer process. If there are differences between the cytosolic Cl^- concentrations ($[Cl^-]_c$) of primary and metastatic tumor cells due to ionic environments surrounding primary and metastatic tumor cells, and the activity of Cl^- transporters and/or Cl^- channels of primary and metastatic tumor cells, the change in $[Cl^-]_c$ of primary and metastatic tumor cells would be a candidate causing the de-adhesion and adhesion. Therefore, we investigated the effect of $[Cl^-]_c$ on the cell-matrix adhesion and the expression of cell adhesion molecules in several gastrointestinal tumor cell lines. Our study indicates that cytosolic Cl^- is a key factor regulating expressions of cell adhesion molecules, CD44 and EpCAM involved in tumor invasion, strongly suggesting that changes of $[Cl^-]_c$ would play important roles in invasions and metastasis of gastrointestinal tumor cells.

(COI: No)

S36-6

Molecular mechanism for morphological and functional regulation by scaffold proteins in the bile duct epithelium

Hatano, Ryo; Asano, Shinji (*Dept Mol Physiol, Col Pharm Sci, Ritsumeikan Univ, Shiga, Japan*)

Secretin dependent biliary secretion of ions and water by transporters and/or channels is essential for the regulation of biliary flow. Cystic fibrosis transmembrane conductance regulator (CFTR) plays a key role in the chloride secretion into the bile. In CF patients, totally 5 to 10% of patients develop the progressive biliary fibrosis. ERM (ezrin-radixin-moesin) proteins are identified as cross-linkers between the plasma membrane proteins and actin cytoskeleton. Ezrin interacts with Na^+/H^+ exchanger regulatory factor-1 (NHERF1) via its N-terminal binding domain and with actin cytoskeleton via its C-terminal actin-binding domain. CFTR is associated with NHERF1 via its c-terminal PDZ binding motif. In liver, Ezrin is exclusively expressed in the cholangiocytes and colocalizes with CFTR and NHERF1 at apical membrane of cholangiocyte. In the present study, we have found that ezrin knockdown (*Vil2*$^{kd/kd}$) mice develop severe hepatic injury characterized by extensive bile duct proliferation, periductular fibrosis, and intrahepatic bile acid accumulation. In these mice, apical membrane localizations of CFTR and NHERF1 were disturbed in the bile ducts. Reduced surface expression of these proteins was accompanied by reduced CFTR-mediated Cl^- efflux activity. Our data suggest that ezrin plays essential roles in the regulation of the bile duct morphology and functions.

(COI: No)

Symposium 37

Developmental insights into cellular communications during organogenesis

(March 22, 16:00～17:30, Room E)

S37-1

Linx: a transmembrane protein directing the establishment of neural circuits in the central and peripheral nervous systems during development

Mandai, Kenji (*Grad.Sch.Med.Kobe Univ., Kobe, Japan*)

The establishment of neural circuits relies on neuronal responses to guidance cues that are expressed spatially and temporally in a right place. To address molecular mechanisms underlying axonal growth, guidance, and target field innervation of developing neurons, we performed genome-wide screens and identified a novel gene coding a LIG family transmembrane protein, Linx. Linx forms complexes with receptor tyrosine kinases, TrkA and Ret, to control axonal extension, branching, and guidance of somatosensory and spinal motor neurons. In the brain, Linx is robustly expressed on corticofugal axons, but not on thalamocortical axons. The mice with a null mutation of Linx exhibit a complete absence of the internal capsule, although layer V cortical neurons and thalamic neurons are intact. Moreover, regional inactivation of Linx either in the prethalamus and lateral ganglionic eminence or in the neocortex leads to a failure of internal capsule formation. Furthermore, Linx binds to thalamocortical projections and promotes their outgrowth. Thus, Linx guides the extension of thalamocortical axons in the ventral forebrain and it mediates reciprocal interactions between thalamocortical and corticofugal axons at the pallial-subpallial boundary and guidance and extension of all ascending and descending projections of the mammalian neocortex. These observations indicate that Linx directs the establishment of neural circuits in both the central and peripheral nervous systems during development.

(COI: No)

S37-2

Crosstalk between hair follicle stem cells and their niche

Fujiwara, Hironobu (*RIKEN CDB, Kobe, Japan*)

It is well established that the special microenvironment for stem cells, the stem cell niche, sends signals to stem cells to regulate their behaviour. However, our recent studies indicate that stem cells themselves also act as a niche for neighbouring cells in the epidermal stem cell system. The hair follicle bulge in the epidermis associates with the arrector pili muscle that is responsible for the formation of goosebumps (Torihada in Japanese). We have recently demonstrated that mouse hair follicle bulge stem cells specialize the basement membrane in their niche, by depositing basement membrane protein nephronectin, thereby creating a special microenvironment for the development and anchorage of arrector pili muscles. We propose that bulge stem cells function as tendon cells in providing a physical connection for the muscles. The immobility of bulge stem cell compartment assures that muscle attachment is stable regardless of the stage of hair generation cycle. We expanded our study to different dermal cell populations and found that the epidermal stem cells also instruct morphogenesis and regeneration of skin dermal adipocytes. Periodical activation of epidermal Wnt/beta-catenin signalling during hair follicle morphogenesis and regeneration induces and synchronises the differentiation of adipocytes via secretion of adipogenic factors. Our findings indicate that the epidermal stem cells not only contribute to epidermal homeostasis and regeneration, but also act as unique environments for dermal cell populations to achieve coordinated skin morphogenesis and regeneration.

(COI: No)

S37-3

Evolution of the turtle shell: insights from developmental, paleontological and genomic perspectives

Kuratani, Shigeru (*Evolutionary Morphology, RIKEN CDB, Japan*)

The turtle shell consists of the dorsal half, or the carapace, which is formed of expanded ribs and the thoracic vertebral column, and the ventral dermal moiety, the plastron. The carapace represents an evolutionary novelty, since it shows an unusual topography. During the turtle development, growth of the turtle ribs is arrested in the axial part of the body only allowed to grow laterally towards the carapacial ridge (CR), a turtle-specific embryonic ridge, folding the body wall medially to encapsulate the scapula. The embryonic pattern of the turtle before this folding resembles the recently discovered fossil species, Odontochelys. The CR supports fan-shaped patterning of the ribs by specific expression of some regulatory genes apparently downstream of Wnt signaling. Analysis of draft genomes of Pelodiscus sinensis and Chelonia mydas confirmed a close relationship of turtles to the bird/crocodilian lineage, which split about 250 mya. Embryonic transcriptome analysis revealed an hourglass-like divergence between turtle and chicken embryogenesis, with maximal conservation around the vertebrate phylotypic period. Survey of the P. sinensis genome also allowed us to identify Wnt5a as the only Wnt ligand in the CR, apparently supporting the possible co-option of limb developmental program in the acquisition of the shell. However, our recent RNA^seq analyses sugegst that CR was more likely to have been obtained by modifying the proximal part of the lateral body wall.

(COI: No)

S37-4

On the origin of parasympathetic ganglia

Brunet, Jean-François (*IBENS*)

Neural crest cells migrate extensively and give rise to most of the peripheral nervous system. The formation of sympathetic, enteric, and dorsal root ganglia has been extensively documented. Much less information is available concerning the way in which parasympathetic ganglia form at numerous locations close to their target organ. I will present how parasympathetic precursors, in the form of Schwann Cell Precursors that coexpress the pan-autonomic transcriptional determinant Phox2b, invade the preganglionic branches of cranial nerves, accumulate at their tip and differentiate into the constituent neurons of their targets, the parasympathetic ganglia — a parsimonious solution to the wiring of autonomic pathways.

(COI: No)

Symposium 38

Anatomical and physiological approaches reveal the mechanism of memory retrieval in the Parabrachial Nucleus

(March 22, 16:00～17:30, Room F)

S38-1

Respiratory circuit in Parabrachial nucleus complex might involve in Panic disorder

Arata, Akiko (*Dept Physiol, Hyogo College of Med., Hyogo, Japan*)

The parabrachial nucleus complex (PB) of the pons is known as a respiratory modulating center and autonomic relay nucleus. The PB projected to paraventricular nucleus and amygdala controlling emotion and stress. The distribution of orexinergic axon fibers existed in the PB of neonatal rat. Orexin plays an essential role of establishing sleep-wakefulness cycle besides orexin also contributes to emotional stress and other state-dependent related regulation of ventilation, and the defense response. On the other hand, the PB is considered as an inspiratory termination and chemoreception. We reported previously the PB plays an active inspiratory-expiratory phase switching in neonatal rat. However, the effects of orexin on the relationship between respiration and chemoreception in neonatal stage of the pons had not been investigated. The firing rate of I-E neuron was increased by superfusion of orexin. Under the hypercapnia, orexin induced higher respiratory rate, and long duration of inspiratory activity appeared frequently in the C4 after 10 minutes from orexin application. Orexin increased respiratory rate by facilitating I-E neuron activity in the PB using inspiratory termination as an active phase-switching, and hypercapnia induced more facilitate and repetitive activity in the long duration of inspiratory phase, that kept for a half hour. These results suggested that PB might be involved in hyperventilation syndrome and panic disorder by controlling respiration using inspiratory termination as an active phase-switch under stress condition.

(COI: No)

S38-2

Pathway from the parabrachial nucleus to the phrenic nucleus is activated by hypercapnia

Yokota, Shigefumi[1]; Kaur, Satvinder[2]; Vanderhorst, Veronique G[2]; Saper, Clifford B[2]; Oka, Tatsuro[1]; Yasui, Yukihiko[1]; Chamberlin, Nancy L[2] ([1]*Dept. of Anat. & Morphol. Neurosci., Shimane Univ. Sch. of Med., Izumo, Japan;* [2] *Neurol., BIDMC & Harvard Med. Sch., Boston, USA*)

Elevated CO2 (hypercapnia) facilitates breathing by increasing the depth and frequency of ventilation. Recently, it is suggested that the parabrachial nucleus (PB) is a key mediator of respiratory facilitation in hypercapnia. Our previous studies demonstrated inspiratory facilitation after stimulation of the lateral PB and Kolliker-Fuse nucleus (KF) and the existence of glutamatergic pathways directly and indirectly via the ventrolateral medulla (VLM) from the KF to the phrenic nucleus (PhN). In this symposium, we first show the distribution of PB neurons that are activated by hypercapnia using Fos-immunohistochemistry. After 2 hours exposure to normoxic hypercapnia (10% CO2), a greater number of Fos-immunoreactive neurons were observed in the rostral KF as well as in the lateral crescent (cr), external lateral, and central lateral PB subnuclei compared to the control. Most of these neurons in the PB were positive for VGLUT2 mRNA but not for GAD67 mRNA. Using retrograde tracing combined with Fos-labeling, we secondly show that numerous hypercapnia-activated neurons in both the KF and the cr subnucleus or solely in the KF were labeled following cholera toxin b subunit injected into the VLM and into the PhN, respectively. These findings suggested that glutamatergic PB neurons activated by hypercapnia contribute to diaphragmatic contraction, thereby increasing ventilation through their direct and indirect pathways to the PhN.

(COI: No)

S38-3

Optogenetic demonstration of direct inputs from the lateral parabrachial nucleus to the nociceptive amygdala

Sugimura, Yae K[1,2]; Takahashi, Yukari[1]; Watabe, Ayako M[1]; Kato, Fusao[1] ([1]*Dept Neurosci, Jikei Univ Sch Med, Tokyo Japan;* [2]*Research Fellow of Japan Society for the Promotion of Science*)

A large majority of neurons in the superficial layer of the dorsal horn project to the lateral parabrachial nucleus (LPB). The LPB neurons then project to the capsular part of the central amygdala (CeC), a key structure underlying nociception-induced emotional responses. It is demonstrated that LPB-CeC synaptic transmission is enhanced in various pain models by using electrical stimulation of the fibers arising from the LPB in brain slices. However this approach has limitations in examining direct monosynaptic connections devoid of contamination of synaptic inputs from locally stimulated neurons and fibers arising from other structures. To overcome these limitations, we transfected AAV vector for channelrodopsin (ChR2) expression to the LPB in rats and prepared brain slices containing amygdala with ChR2-expressing fibers 5-7 weeks after transfection. We found that blue light stimulation resulted in EPSCs with very small latency fluctuation and potent polysynaptic feed-forward inhibition in CeC neurons regardless of firing pattern type. Intraplanter formalin injection made 24 hours before the slice preparation resulted in a significantly larger EPSC amplitude than those with saline injection only in the CeC neurons showing late-firing pattern. These results suggest that direct inputs from the LPB are enhanced only in a specific type of CeC neurons in inflammatory pain model.

(COI: No)

S38-4

Genetic tracing reveals the architectural solution in the parabrachial nucleus that processes taste information and gates emotional memory

Sugita, Makoto; Yamamoto, Kuniyo; Hirono, Chikara; Shiba, Yoshiki (*Department of Physiology and Oral Physiology, Institute of Biomedical & Health Sciences, Hiroshima University, Hiroshima, Japan*)

Bitter and sweet taste stimuli elicit contrastive behavioral and emotional responses. Therefore, the gustatory system provides a simple model to investigate neuronal mechanisms underlying behavioral and emotional responses, and learning. We used a genetic approach to visualize the neuronal circuitries of bitter and sweet taste processing by expressing the fluorescently labeled transneuronal tracer, tWGA-DsRed, in either bitter- or sweet-responsive taste receptor cells in mice. The spatial distribution of neurons labeled by tWGA-DsRed that originated from taste receptor cells suggests that gustatory neurons dispersed in the solitary tract nuclei, the parabrachial nuclei, the thalamic gustatory area, and the gustatory cortex may be organized with sweet inputs located rostral and with bitter inputs located caudal, except for bitter inputs into the external-lateral and external-medial subdivisions of the parabrachial nuclei, and the complex inputs in the amygdala. The tracer-labeled neurons in the parabrachial nuclei were further characterized by electrophysiological and immunohistochemical analyses, and mapping the induction of the immediate early gene by taste stimuli and aversion-eliciting stimuli from the gut. Our data suggest that the different types of taste-relaying neurons are clustered in distinct locations, showing the architectural solution in the parabrachial nucleus that processes taste information and gates emotional memory.

(COI: No)

S38-5

Parabrachial nucleus is a center of emotional expressions

Ohmura, Yoshiyuki; Kuniyoshi, Yasuo (*Dept. Mechano-Infomatics, Grad Sch Info and Tech., Univ. of Tokyo, Tokyo, Japan*)

The parabrachial nucleus (PB) has a great variety of connections. The PB receives inputs from Lamina I of spinal and trigeminal dorsal horns, nucleus of solitary tract, vestibular nucleus, area postrima, superior colliculus, inferior colliculus, periaqueductal gray(PAG), hypothalamus, amygdala, the bed nucleus of the stria terminalis(BNST), insular cortex, cerebellum. And PB projects to the nucleus raphe magnus, the reticular formation, the motoneurons controlling the diaphragm, the jaw closers, perioral musculature, orbicularis oculi, the tongue protruders, the pharynx, larynx and esophagus. All of these projections are reminiscent of the relations with emotional expressions. In a hierachical view of central nervous system, hypothalamus is a higher center of homeostatic control and PAG is a more complex modulator of social responses. However, because the projections of PB are generally reciprocal, we can make a hypothesis that PB will be a higher center of emotional expressions, PAG will be a reflex circuit for reproduction and hypothalamus will be a reflex circuit for endocrine secretion. Although the PAG has stronger connections with superior colliculus and inferior colliculus, the projections are limited to the regions related to reproduction (nociception, micturition and vocalization). The PB seems to have broader projections. CGRP inhibition neurons from PB potentiate anxiety-like behaviors and appetite suppressions. The PB will have a switch control between innate motions and voluntary motions, and relate to memory retrieval by activation of cerebral cortex.

(COI: No)

Symposium 39

Frontier researches on the suprachiasmatic nucleus, the center of the mammalian circadian timing system

(March 22, 16:00～17:30, Room G)

S39-1

Mechanisms of circadian rhythm generation in the suprachiasmatic nucleus of Cry1/2 deficient mice

Ono, Daisuke[1]; Honma, Ken-ichi[2]; Honma, Sato[2] ([1]*Photonic Bioimaging Section, Hokkaido Univ, Grad Sch of Med, Sapporo, Japan;* [2]*Department of Chronomedicine, Hokkaido Univ, Grad Sch of Med, Sapporo, Japan*)

In mammals, the circadian pacemaker is located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Circadian rhythms are generated by transcription and translation autoregulatory feedback loop involving several clock genes, such as *Pers*, *Crys*, *Bmal1*, and *Clock*, in which *Cry1* and *Cry2* play essential roles. However we recently reported circadian rhythms in the SCN slice of *Cry1/Cry2* double deficient ($Cry1^{-/-}/Cry2^{-/-}$) mice in neonatal period. The rhythms disappeared in adults due to desynchronyzation of cellular rhythms. In addition, exposure $Cry1^{-/-}/Cry2^{-/-}$ mice to constant light during neonatal period restored circadian behavioral rhythms in adult hood. It is still unclear how the rhythm is generated without *Cry1/Cry2*. Interlocking with the above mentioned feedback loop, *Dec1* and *Dec2* consists another feedback loop and suppress transcriptions of *Pers* and *Crys* through binding upstream E-box enhancers. *Dec1/Dec2* could be involved in the circadian rhythm generation in an absence of *Cry1/Cry2*. To examine the compensatory role of *Dec1/Dec2* in the $Cry1^{-/-}/Cry2^{-/-}$SCN, we made mice lacking these 4 genes by crossing $Cry1^{-/-}/Cry2^{-/-}$ mice and $Dec1^{-/-}/Dec2^{-/-}$. The results showed that *Dec1/Dec2* could not compensate *Cry1/Cry2*. However, they involved in determining oscillation speed. We will discuss the roles of molecular feedback loops involving *Dec1/Dec2*.

(COI: No)

S39-2

Molecular and neuronal mechanisms underlying jet lag

Yamaguchi, Yoshiaki; Okamura, Hitoshi (*Grad.Sch.Pharm.Kyoto Univ., Kyoto, Japan*)

Circadian clock is an essential biological property that coordinates behavioral, physiological, and metabolic systems. Generally, we are not aware of our biological clock process since it is completely synchronized with external light-dark (LD) cycles. However, travelling rapidly across multiple time zones makes us aware of desynchrony between internal clock and the environmental time, resulting in sleep disorder and gastrointestinal distress. Moreover, recent works have reported that repeated jet-lag exposure and rotating shift work increase the risk of cancer and metabolic insufficiency. Although jet lag is considered as a chronobiological problem, the molecular and neuronal mechanisms are poorly understood. Here, we show that mice genetically deficient in vasopressin V1a and V1b receptors (V1aV1bDKO) are resistant to jet lag. Circadian rhythms of locomotor activity, clock gene expressions, and body temperature rapidly re-entrained to phase-shifted LD cycles in V1aV1bDKO mice. Nevertheless, the behavior of V1aV1bDKO mice was still coupled to the internal clock, which oscillated normally under standard LD and DD conditions. Real-time imaging of hundreds of cellular rhythms in the suprachiasmatic nucleus (SCN, the master clock) suggested that V1a/V1b-mediated cell-cell communication confers on the SCN an intrinsic resistance to external rhythm perturbation. Pharmacological inhibition of V1a and V1b in the SCN of wild-type mice accelerated the speed of recovery from jet lag, which promises vasopressin signaling as a pharmaceutical target for jet lag and shift work-related diseases.

(COI: No)

S39-3

Towards Organisms-level Systems Biology

Ueda, Hiroki R (*Graduate School of Medicine, The University of Tokyo*)

The logic of biological networks is difficult to elucidate without (1) comprehensive identification of network structure, (2) prediction and validation based on quantitative measurement and perturbation of network behavior, and (3) design and implementation of artificial networks of identified structure and observed dynamics.Mammalian circadian clock system is such a complex and dynamic system consisting of complicatedly integrated regulatory loops and displaying the various dynamic behaviors including i) endogenous oscillation with about 24-hour period, ii) entrainment to the external environmental changes (temperature and light cycle), and iii) temperature compensation over the wide range of temperature. In this symposium, I will discuss the current and past studies on a mammalian circadian clock as an example of molecule-to-cell-level systems biology, and introduce two design principles, which may underlie biological timings. I would also like to discuss about the challenges and opportunities towards the organism-level systems biology.

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(COI: No)

S39-4

Cellular Circadian Oscillators in Vasopressin Neurons of the Suprachiasmatic Nucleus Play a Critical Role in Coupling between Morning and Evening Behavioral Rhythms in Mice

Mieda, Michihiro (*Dept Molecular Neuroscience and Integrative Physiology, Fac Med, Kanazawa Univ, Kanazawa, Japan*)

The suprachiasmatic nucleus (SCN) is the primary circadian pacemaker in mammals and entrains to the environmental light/dark cycle. It is composed of multiple types of neurons, and neuronal network properties are integral to normal function of the SCN. However, mechanisms underlying the SCN neuronal network have remained elusive. As a first step to understand the principle of the SCN network, we generated mice in which *Bmal1*, an essential clock component, is deleted specifically in the neurons producing arginine vasopressin (AVP), one of the primary neuronal types in the SCN ($Avp\text{-}Bmal1^{-/-}$ mice). $Avp\text{-}Bmal1^{-/-}$ mice showed marked lengthening in the free-running period and activity time of behavior rhythms. When exposed to an abrupt 8 hr advance of the light/dark cycle, $Avp\text{-}Bmal1^{-/-}$ mice reentrained faster than control mice did. In $Avp\text{-}Bmal1^{-/-}$ mice, the circadian expression of genes involved in intercellular communications, including *Avp*, *Prokineticin 2*, and *Rgs16*, was drastically reduced in the dorsal SCN, where AVP neurons predominate. In slices, dorsal SCN cells showed attenuated PER2::LUC oscillation with highly variable and lengthened periods. Thus, *Bmal1*-dependent oscillators of AVP neurons may modulate the coupling of the SCN network, eventually coupling morning and evening behavioral rhythms, by regulating expression of multiple factors important for the network property of these neurons.

(COI: No)

S39-5

Structures that deliver the circadian rhythm from the suprachiasamtic nucleus to neighboring brain regions

Masumoto, Kohei; Nagano, Mamoru; Koinuma, Satoshi; Sujino, Mitsugu; Shigeyoshi, Yasufumi (*Dept. of Anat. and Neurobiol. Kinki Univ. Sch. Med., Osaka, Japan*)

The suprachiasmatic nucleus (SCN) is the center of circadian clock. It has been known that the brain region neighbouring the SCN indicates circadian rhythm synchronized with that in the SCN, what underlying mechanism transfer the circadian rhythm to neighboring brain regions has been obscure. The paraventricular nucleus (PVN) and subparaventricular zone (SPZ) are located in the dorsal to the SCN, and have been known to relay the circadian rhythm phase of the SCN to the other brain regions. In order to delineate what biological structure transmits circadian rhythm to the PVN/SPZ from the SCN, we observed the coherence between the circadian rhythms in the SCN and PVN/SPZ by monitoring bioluminescence emitted from tissue slice from neonatal Per2::luc knock-in mice. In slices containing SCN and PVN/SPZ, the two regions showed antiphasic circadian rhythm, in contrast, slices containing PVN/SPZ but not SCN showed a circadian rhythm which was damped after a few days. However, when the slice showing damped oscillation was co-cultured with the SCN slice, PVN/SPZ restored a stable circadian rhythm antiphasic to that in the SCN about one week after the treatment. The findings suggest that the structure maintaining the coherence between the circadian oscillations in the SCN and PVN/SPZ was reconstructed.

(COI: No)

Symposium 40

Variety in neural circuit construction and underlying principles

(March 22, 16:00～17:30, Room H)

S40-1

Molecular and cellular basis for establishment and remodeling of dendritic fields

Emoto, Kazuo (*Grad.Sch.Sci., Univ.of Tokyo, Japan*)

The refinement of neural circuits involves dendrite pruning, a process to remove inappropriate projections that are formed during neural development. In Drosophila sensory neurons, compartmentalized calcium (Ca2+) transients in dendritic branches act as temporal and spatial cues to trigger pruning, yet how neurons define the dendritic branches with Ca2+ transients remains elusive. Here we report that local endocytosis in proximal dendrites induces the compartmentalization of the Ca2+ transients. Live imaging of single dendrites revealed a massive increase of endocytic activity in proximal dendrites that spatially and temporally correlates with dendrite thinning, an early step in pruning tightly coupled with compartmentalized Ca2+ transients. We identified two GTPases, Rab5 and dynamin, as critical regulators of the local endocytosis in proximal dendrites; blocking the activity of these GTPases prevented dendrite thinning and impaired the occurrence of compartmentalized Ca2+ transients. These data indicate that local endocytosis drives dendrite thinning in the proximal dendrites to promote compartmentalized Ca2+ transients.

(COI: No)

S40-2

Energy homeostasis in growing dendrites of cerebellar Purkinje cells

Kengaku, Mineko[1,2]; Fukumitsu, Kansai[1,2]; Fujishima, Kazuto[1]; Hatsukano, Tetsu[1,2] ([1]*WPI-iCeMS, Kyoto Univ., Japan;* [2]*Grad.Sch.Biostudies, Kyoto Univ., Japan*)

The highly branched dendrites of vertebrate CNS neurons possess huge volumes and surface areas, necessitating robust and specific mechanisms for maintaining proper homeostatic control of their intracellular environment. We have studied molecular and cellular mechanisms underlying energy homeostasis in growing dendrites of cerebellar Purkinje cells. We found that local ATP synthesis by dendritic mitochondria, at least when it is supported by the ATP buffering activity of creatine kinases, is required to maintain the ATP levels that dendrites need for their continuous outgrowth, both in vitro and in vivo. Additionally, our results suggest that actin turnover and organization, as regulated by the ATP-dependent phosphorylation cycle of ADF/cofilin, plays an important role in feedback control of ATP consumption during dendritic outgrowth.

(COI: No)

S40-3

Molecular composition and functional mechanism of AMPA receptor complexes

Nakagawa, Terunaga (*Vanderbilt University Medical Center, Nashville, TN, USA*)

AMPA-type ionotropic glutamate receptors (AMPARs) mediate the majority of excitatory synaptic transmission and their dysfunction involves a variety of neurological and psychiatric disorders. Understanding the molecular mechanism of AMPAR function is thus critical for continued development of new therapeutic agents that targets AMPARs and to understand the molecular basis of synaptic plasticity and neural circuit function. The majority of the AMPARs in the brain form complex with auxiliary factors, including TARPs, CNIHs, synDIG1, CKAMP44, and GSG1L. Auxiliary subunits do not constitute the channel pore but physically interact with pore forming alpha subunits of AMPAR, GluA1-4. Because they regulate AMPAR trafficking and gating, loss and gain of function of AMPAR auxiliary subunits impact synaptic function in animal models. Different auxiliary subunits of AMPA-Rs modulate receptor function in specific ways. Combinatorial effects of four GluA subunits binding to various auxiliary subunits amplify the functional diversity of AMPA-Rs. The significance and magnitude of molecular diversity, however, remain elusive. Our goal is to extract principles of auxiliary subunit function by studying their diverse molecular mechanism using cryo-EM and their function using rodent genetic models. Recent progress in our laboratory will be presented.

(COI: No)

S40-4

Molecular and cellular mechanisms underlying learning

Takahashi, Takuya (*Dept Physiol, Sch Med, Yokohama city Univ, Kanagawa, Japan*)

Learning induces plastic changes in synapses. However, the regulatory molecules that orchestrate learning-induced synaptic changes are largely unknown. Although it is well established that cholinergic inputs from the medial septum modulate learning and memory, evidence for the cholinergic regulation of learning-induced synaptic plasticity is lacking. We present that the activation of muscarinic acetylcholine (ACh) receptors (mAChRs) mediates the contextual fear-learning-driven strengthening of hippocampal excitatory pyramidal synapses, through the synaptic incorporation of AMPA-type glutamate receptors (AMPARs). Contextual fear learning also enhances the strength of inhibitory synapses on hippocampal pyramidal neurons, in a manner mediated by the activation of, not mAChRs, but nicotinic AChRs (nAChRs). Interestingly, we observed a significant cross-correlation between the learning-induced increases in excitatory and inhibitory synaptic strength at individual pyramidal neurons. Understanding the mechanisms underlying cholinergic regulation of learning-induced hippocampal synaptic plasticity may help the development of new therapies for cognitive disorders such as Alzheimer's disease.

(COI: No)

Symposium 41

Stem cell therapy for neuronal disorders

(March 22, 16:00～17:30, Room I)

S41-1

A subpopulation of fibroblasts, Muse cells, ameliorate rat stroke model

Morita, Takahiro (*Department of Neurosurgery, Grad.Sch.Med.Tohoku Univ., Sendai, Miyagi, Japan*)

Multilineage-differentiating stress-enduring (Muse) cells, a distinct subpopulation of fibroblasts corresponding to ~1% of the total, exhibit pluripotency in vitro and replace lost cells and repair tissues in vivo. We investigated whether human fibroblast-derived Muse cells facilitate functional recovery in ischemic stroke in rats. Human dermal fibroblasts, separated into stage-specific embryonic antigen-3(+) Muse cells and antigen-3(-) non-Muse cells, were injected into rat brains 2 days after infarction. Histologic and behavioral tests were examined for up to day 87 after transplantation. The Muse-transplanted group exhibited significantly improved neurologic outcomes at the chronic stage of stroke, after day 70, compared with the non-Muse-transplanted group, without a change in the infarct size. Muse cells survived in the host brain and differentiated spontaneously into β-tubulin(+)and GFAP(+) cells by day 87 after transplantation, whereas almost all of the non-Muse cells disappeared from the host brain. Retrograde labeling further revealed that the integrated Muse cells extended their neurites into the brainstem. No tumorigenicity was observed after transplantation. Muse cells possess high potential to spontaneously differentiate into neural lineage cells in the host brain and facilitate functional recovery from stroke. Our results demonstrated that general fibroblasts are strong cell candidates for treating stroke if the Muse cell component is fully utilized by purification or enrichment.

(COI: No)

S41-2

Functional recovery after rat spinal cord injury by tissue regenerating factors derived from mesenchymal stem cells

Yamamoto, Akihito (*Grad.Sch.Med.Nagoya Univ., Nagoya, Japan*)

Human adult dental pulp stem cells (DPSCs) and stem cells from human exfoliated deciduous teeth (SHED) are self-renewing mesenchymal stem cells residing within the perivascular niche of the dental pulp. They are thought to originate from the cranial neural crest, expresses early markers for both mesenchyme and neuroectodermal stem cells and are able to differentiate into the functional neurons and oligodendorocytes under appropriate conditions. Studies have reported that engrafting these pulp stem cells promote functional recovery from various types of acute and chronic CNS insults. Here we show that the intrathecal administration of conditioned serum-free medium (CM) from SHED into the severe adult rat spinal cord injury (SCI) led to a marked recovery of hindlimb locomotor function. SHED-CM treatment inhibited SCI-induced apoptosis, preserved neural fibres and myelin sheaths, and promoted the growth of the descending 5-HT+ axons. Importantly, we show here that these neuroregenerative activities were supported by a marked immunoregulatory function of SHED-CM, by which the pro-inflammatory M1 microglia/macrophages were directly converted to the anti-inflammatory/tissue repairing one. We have identified a novel set of M2-inducer in SHED-CM, which is necessary and sufficient for SHED-CM-mediated M2 induction and functional recovery after SCI. Thus, our data suggest that the anti-inflammatory/tissue-regenerative condition generated by the dental pulp stem cells play a central roles in functional recovery from SCI. I declare that there are no competing interests.

(COI: No)

S41-3

Mesenchymal stem cell therapy of spinocerebellar ataxia type 1 model mice

Nakamura, Kazuhiro; Hirai, Hirokazu (*Dept Neurophysiol, Grad Sch Med, Gunma Univ, Maebashi, Japan*)

Spinocerebellar ataxia (SCA) is a devastating progressive neurodegenerative disorder. Currently, no effective treatments have been developed. However, some studies have shown that an intracerebellar injection of mesenchymal stem cells (MSCs) was partially effective in some mouse models of cerebellar ataxia. MSCs likely exert their therapeutic efficacy by secreting innate factors to induce neuronal growth, synaptic connection and reduce apoptosis. We tested if the factors released from MSCs bring the therapeutic effects for SCA type 1 (SCA1) transgenic mice by intrathecally injecting MSCs conditioned medium into 5 weeks old mice, which was followed by weekly intravenous injections. The conditioned medium successfully reversed the disturbed motor coordination observed using rotarod test. Likewise, the conditioned medium also corrected delayed nerve conduction in the spinal motor neurons of SCA1-knockin mice. Collectively, the unknown factors released from MSCs work to correct functional disturbances seen in SCA1 model mice. We also introduce potential mechanisms by which the unknown factors exert the therapeutic effects on SCA1 mice.There are no potential conflicts of interest in the content of this presentation.

(COI: No)

Symposium 42

Birthplace, birthtime and molecular mechanisms of oligodendrogenesis

(March 22, 16:00～17:30, Room J)

S42-1

Molecular mechanisms underlying the production of cortical oligodendrocytes from neural stem cells

Hitoshi, Seiji (*Dept Integrative Physiol, Shiga Univ Med Sci, Otsu, Japan*)

Oligodendrocyte precursor cells (OPCs) appear in the late embryonic brain, mature to become oligodendrocytes (OLs) and form myelin in the postnatal brain. Recently, it has been proposed that early-born OPCs derived from the ventral forebrain are eradicated postnatally and that late-born OLs predominate in the cortex of the adult mouse brain. However, intrinsic and extrinsic factors that specify the ability of self-renewing multipotent neural stem cells in the embryonic brain to generate cortical OL-lineage cells remain largely unknown. Using an inducible Cre-loxP system to permanently label Nestin- and Olig2-lineage cells and using an in utero electroporation technique, we determined when and where cortical OL-lineage cells differentiate from neural stem cells in the developing mouse brain. We show that neural precursor cells in the dorsal VZ/SVZ are inhibited by Wnt signaling from contributing to cortical OLs in the adult brain. By contrast, neural precursor cells present in the dorsoventral boundary VZ/SVZ produce a significant amount of OLs in the adult cortex. Our results suggest that neural stem cells at this boundary are uniquely specialized to produce myelin-forming OLs in the cortex.

(COI: No)

S42-2

Origin of optic nerve oligodendrocyte in the developing mouse

Ono, Katsuhiko[1]; Ikenaka, Kazuhiko[2] (*[1]Kyoto Pref Univ Med, Kyoto, Japan; [2]Natl Inst Physiol Sci, Okazaki, Japan*)

Oligodendrocytes (OLs) are myelinating cells in the central nervous system (CNS). Oligodendrocyte precursor cells (OPCs), which express Olig2 and PDGFRalpha, are reported to originate in the restricted region of each subdivision in the CNS at early developing stage. In the present study, we examined whether optic nerve OL originate in the basal forebrain in the fetal mouse. In the early stages of the ventral forebrain, Olig2+ or PDGFRalpha+ OPCs were distributed around the third ventricle and first OPCs in the optic nerve appeared at E15.5. High titer retrovirus vector carrying lacZ gene was injected into the lateral and third ventricles of fetal mouse at e12.5, E14.5 or E15.5 at which optic ventricle was disconnected from the third ventricle, and LacZ+ cell distribution was examined in the adult optic nerve. LacZ+ cells with OL-like profiles were observed in 4 optic nerves out of 106 nerves. Once LacZ+ cells were observed in the optic nerve, several hundred cells were distributed in the single nerve. We next used Olig2-CreER/Rosa26-GEFP-reporter double heterozygous mice with tamoxifen treatment at E12.5 or E15.5 and adult optic nerves were examined. In 7 out of 15 animals, EGFP+ cells were observed and some of their processes were PLP+ and some of somata were CC1+. These results clearly demonstrated that optic nerve OPCs are derived from ventral basal forebrain and that they enter the optic nerve at round E15.5, and that they differentiate into mature OL throughout the optic nerve.

(COI: No)

S42-3

Microglia enhance oligodendrogenesis in the early postnatal subventricular zone

Sato, Kaoru (*Lab. Neuropharmacol., Div. Pharmaol., NIHS, Tokyo, Japan*)

Microglia have long been considered as resident immune cells, which are activated in response to pathological events. However, the physiological importance of microglia in the normal CNS has been clarified these days. We recently found a new physiological role of microglia in brain development. We found large numbers of activated microglia in the forebrain subventricular zone (SVZ) of the rat from P1 to P10. Pharmacological suppression of the activation, which produces a decrease in levels of a number of proinflammatory cytokines, i.e., IL-1beta, IL-6, TNF-alpha, and IFN-gamma, significantly inhibited oligodendrogenesis together with neurogenesis in the SVZ. In vitro neurosphere-assays reproduced the enhancement of oligodendrogenesis and neurogenesis by activated microglia and showed that the cytokines revealed the effects complementarily. These results suggest that activated microglia accumulate in the early postnatal SVZ and that they enhance oligodendrogenesis and neurogenesis via released cytokines.

(COI: No)

S42-4

Pathophysiology of oligodendrocyte in multiple sclerosis

Nakahara, Jin (*Dept. of Neurol., Keio Univ., Tokyo, Japan*)

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) affecting nearly 2.5 million people worldwide. It has long been assumed that MS is a T-cell-mediated autoimmune disease targeting CNS myelin, however recent neuropathological and radiological studies suggested that neurodegeneration (i.e. brain atrophy) also occurs in MS, independently of inflammatory demyelination. Although myelin is an exceptional structure that spontaneously regenerates within the CNS, remyelination failure may jeopardize recovery after demyelinating insults in a subset of MS patients. Such failure is attributable in part to the degeneration of preserved oligodendrocytes in MS lesions, together suggesting inflammatory demyelination, brain atrophy and "oligodendrogliopathy" are key pathologies in the disease. Understanding the pathophysiology of oligodendrocytes in MS is essential to overcome the disease, especially for the future remyelination strategy. In this presentation, the pathophysiology of oligodendrocytes in MS will be reviewed and possible remyelination strategies will be discussed.

(COI: Properly Declared)

Symposium 43

Generation of Physiological Functions During Ontogenesis : Looking for the Frontier of "Functiogenesis"

(March 22, 17:30～19:00, Room E)

S43-1

Functiogenesis of the embryonic CNS revealed by multiple-site optical recording with a voltage-sensitive dye

Sato, Katsushige[1]; Momose-sato, Yoko[2] (*[1]Dept Hlth & Nutr Sci, Fac Human Hlth, Komazawa Women's Univ, Tokyo, Japan; [2]Dept Hlth & Nutr, Coll Human Enviro Studies, Kanto-Gakuin Univ, Yokohama, Japan*)

The functiogenesis of the embryonic central nervous system has long been unclear, because conventional electrophysiological means have several technical limitations. First, early embryonic neurons are small and fragile, and the application of microelectrodes is often difficult. Second, the simultaneous recording of electrical activity from multiple sites is limited, and as a consequence, response patterns of neural networks cannot be assessed. We have applied optical recording techniques with voltage-sensitive dyes to the embryonic central nervous system and provided a new approach to the analysis of the functiogenesis of the central nervous system. In this symposium, we present recent progress in optical studies on the embryonic central nervous system with special emphasis on development of the olfactory system. The studies clearly demonstrate the utility of voltage-sensitive dye imaging as a powerful tool for elucidating the functional organization of the vertebrate embryonic central nervous system.

(COI: No)

S43-2

Perinatal modulations of the respiration-related rhythmic activities by GABA and Cl^- co-transporters

Okabe, Akihito; Shimizu, Chigusa; Takayama, Chitoshi (*Dept. Mol. Anat., Sch. Med., Univ. of the Ryukyus, Okinawa, Japan*)

GABA is one of main inhibitory neurotransmitter in adult central nervous system but an excitatory neurotransmitter during early postnatal development. Such GABA action shift from excitatory to inhibitory is caused by decreasing of intracellular chloride concentration ($[Cl^-]_i$) which is determined by balance of K^+-Cl^- co-transporter-extrusion system (KCC2) and Na^+, K^+-$2Cl^-$ co-transporter-accumulation system (NKCC1). Role of GABAergic transmission in regulation of medullary respiration-related rhythmic activity (RRA) perinatally, however, is yet to be determined. Here, we examined how GABA and chloride co-transporters contribute to RRA during development in hypoglossal nucleus (12N) where inspiratory neurons reside. We recorded extracellular RRA in medullary slices obtained from embryonic day (E) 16 to postnatal day (P) 7 mice. RRA was induced by soaking slices in artificial cerebrospinal fluid (aCSF) containing 8 mM-K^+. Mean numbers of RRA were significantly increased from E16 to P0 but there were no significant changes in RRA during postnatal development. Application of GABA significantly decreased frequency of RRA on E16 but increased it after P3, whereas application of DIOA, a KCC2 blocker, significantly increased frequency of RRA on E16 but significantly decreased it after P1. In addition, dense KCC2 immunolabeling was seen in 12N from E16 and P7. These results suggest that decreasing $[Cl^-]_i$ levels caused by increasing KCC2 levels in 12N could play important roles in regulating the frequency of RRA during development.

(COI: No)

S43-3

GABA and glycine evoke depolarizing responses in early neonatal rat CNS

Ito, Susumu; Cherubini, Enrico (*[1]Grad Sch Emerg Med Sys, Kokushikan Univ, Tokyo, Japan; [2]Euro Brain Res Inst, Rome, Italy*)

GABA and glycine are the main inhibitory neurotransmitters in the adult CNS. Both GABA and glycine bind to receptors coupled to chloride channels. In adult neurons, the level of intracellular chloride is maintained at relatively low levels and E_{Cl^-} is below the resting membrane potential (V_m). Therefore, GABA and glycine hyperpolarize the membrane and inhibits neuronal firing through an inwardly directed flux of chloride. In neonatal animals, intracellular chloride is relatively high, and E_{Cl^-} is above V_m. Therefore, GABA and glycine depolarizes the membrane through an outwardly directed efflux of chloride. To be excitatory, GABA- and glycine-mediated membrane depolarization should reach the threshold for action potential generation. The intracellular chloride concentration is under control of two main cation-Cl^- co-transporters the NKCC1 and KCC2 that import and export $[Cl^-]_i$ respectively. The unbalance between these two transporters is responsible for the high $[Cl^-]_i$ found early in postnatal life. The developmentally up-regulated expression of the K^+/Cl^- co-transporter KCC2 is responsible, toward the end of the first postnatal week, for the shift of GABA and glycine from the depolarizing to the hyperpolarizing direction. In the immature hippocampus, the synergistic action of glutamate and GABA, both depolarizing and excitatory, triggers coherent network oscillations, the so-called giant depolarizing potential or GDPs. GDPs associated calcium transients are instrumental for enhancing synaptic activity at emerging GABAergic and glutamatergic pathways.

(COI: No)

S43-4

Optical assessment of ontogenic origin of vertebrate cardiac pacemaker functions : A cultured multiple-hearts study

Sakai, Tetsuro[1,2]; Kamino, Kohtaro[2] (*[1]Dept. Systems Physiol. Univ. of the Ryukyus Grad. Sch., Okinawa, Japan; [2]Tokyo Med. Dent. Univ., Tokyo, Japan*)

To elucidate the functional organization of cardiac pacemaker, we have used the early stage chick embryos with multiple-hearts which were made experimentally in whole embryo culture and examined the spatial gradient of intrinsic ryhthmicity using optical methods. The embryos were cut microsurgically through the tissue of the anterior intestinal portal at the 5- to early 7-somite developmental stage. Spontaneous electrical activity in 4 to 6 segmented hearts, during the 7- to 10-somite stages of development, were monitored simultaneously by means of multiple-site optical recordings of membrane potential activity, using a voltage-sensitive. Each segment of the heart exhibited its own inherent rhythmicity. In quadruple-hearts, the order of the rhythmicity was often [left-caudal segment] > [right-caudal segment] > [left-cephalic segment] > [right-cephalic segment]; the heart rate in the left-caudal segment was often faster than that in the other segments. These findings strongly emphasize the concept that, in the early phases of cardiogenesis, the formation of a regional gradient of pacemaker activity (i.e. a spatial gradient of intrinsic rhythmicity) results in the functional self-organization of the pacemaking area. And, in intact embryonic chick and rat hearts, we confirmed that this concept agrees the early process of the functional organization of the cardiac pacemaker that we recorded optically.

(COI: No)

Symposium 44

Impacts of active experience on brain morphology and function

(March 22, 17:30～19:00, Room F)

S44-1

Maternal experiences improve spatial learning through hippocampal neural plasticity

Furuta, Miyako; Fukushima, Atsushi; Funabashi, Toshiya; Akema, Tatsuo (*Department of Physiology, St. Marianna University School of Medicine, Kawasaki, Japan*)

Maternal experiences consist of a series of events including pregnancy, delivery, lactation and rearing. We questioned if those events can alter the behavior and the neural system of the mother rats. Behavioral changes during postpartum period are well documented in rats. The main factor is considered to be a drastic reduction of estrogen levels after delivery. In this study postpartum rats that resumed estrus cycle after weaning, and nulliparity controls at the same age, were subjected to Morris water maze and Y-maze tests for spacial learning assessments. In electrophysiological study, we used a protocol for inducing long-term potentiation using whole-cell recording to pair low-frequency synaptic stimulation (270 pulses, 10 Hz) with a depolarizing voltage-clamp pulse (1.5 min duration). Furthermore, the expression level of GluR1 and R2 was analyzed in the hippocampus by western blot using synaptosome fraction. Improved performance of primiparous rats was found in Y maze. The expression of GluR2 level was increased in the hippocampus of primiparous rats. Taken together, reproductive experience could change hippocampal function leading to improve spatial learning.

(COI: No)

S44-2

Rearing in enriched environment induces beneficial alterations in the central nervous system and emotional behavior

Urakawa, Susumu[1]; Ono, Taketoshi[1]; Nishijo, Hisao[2] (*[1]Dept Neurophysiotherapy, Grad Sch Med Pharmaceu Sci, Univ Toyama, Japan; [2]Dept System Emotional Science, Grad Sch Med Pharmaceu Sci, Univ Toyama, Japan*)

Early life experiences can modulate development of the neuronal network and modify various behaviors. Rearing in an enriched environment (EE) is generally believed to facilitate enhanced motor, sensory, and cognitive stimulation and also provide relatively increased social interaction than a standard environment (SE; housing conditions in conventional laboratory cages). In our previous reports, EE rats showed various alterations in central nervous system and behavioral outcome. The direct striatal injection of neuro-toxin caused neuronal cell-loss and subsequently occurred cell-replacement. However, EE ameliorated lesion-induced motor dysfunction and increased the number of migrating cells in the lesioned-striatum. These results suggest that EE induces beneficial effects in neuronal cell-replacement and recovery from motor dysfunction. In addition, we reported that EE significantly affected emotional responses and the number of parvalbumin-positive inhibitory neurons in the amygdala. The EE rats showed a decrease in anxiety-like behavior in the open field and high performance in a beam walking test. Compared with SE rats, EE males showed decreased sexual activity. The number of parvalbumin-positive neurons in the basolateral amygdala was increased by EE, correlated with performance in the beam walking behavior. The results suggest that EE induced behavioral plasticity, which might be mediated through its effects on parvalbumin-positive neurons.

(COI: No)

S44-3

An increase/decrease in physical activity influences structural and functional adaptive changes in the hippocampus

Nishijima, Takeshi; Kamidouzono, Yoshika; Ishiizumi, Atsushi; Kita, Ichiro (*Dept Human Health Sci, Tokyo Metropolitan Univ, Tokyo, Japan*)

The hippocampus is a highly plastic part of the brain that adaptively responds to levels of physical activity. A higher level of physical activity is a key factor in promoting structural and functional improvements of the hippocampus. The hippocampus has a functional gradient along its dorso-ventral axis; the dorsal part of the hippocampus plays a key role in spatial learning and memory, whereas the ventral hippocampus is involved in regulating emotional behaviors. Importantly, behavioral studies indicate that exercise can not only improve spatial learning and memory but also exert anxiolytic and antidepressant effects in rodents. Recently, we have shown that long-term exercise induces delta-FosB expression, a marker for chronic neuronal activation, and enhances neurogenesis throughout the dorso-ventral axis of the hippocampus. These findings support the current understanding that exercise is an effective non-pharmacological intervention that can improve multiple hippocampal function. It is also important to understand how a reduction in physical activity, i.e. a lack of positive experience, deteriorates hippocampal function. We found that the forced physical inactivity, by cessation of voluntary wheel running, was anxiogenic and impaired hippocampal neurogenesis. Although further studies are needed to confirm our findings, our results propose a new hypothesis that physical inactivity can be a risk factor for stress-induced mood disorders, which may be in part caused by the reduction of hippocampal function.

(COI: No)

S44-4

Neuroendocrine correlates of maternal behavior in humans and its developmental changes during pregnancy to motherhood

Nishitani, Shota; Doi, Hirokazu; Takamura, Tsunehiko; Shinohara, Kazuyuki (*Dept. Neurobiol. Behav., Grad. Sch. Biomedical Sci., Nagasaki Univ. Nagasaki, Japan*)

Maternal behavior has been recognized as to be essential for child development and mental health. To investigate the biological vulnerability to child rearing problems, researchers have been devoted to identify the neural correlates in humans. Although substantial amount of studies have showed possible neural correlates of maternal behaviors, there is little agreement on the link between the neural correlates and hormones so far. In voles, however, whole picture of molecular substitutes for maternal behavior has been shown. Comparing molecules and genes between prairie and mountain voles, it has been established that maternal behavior are closely associated with oxytocin receptor (OXTR). However, the influences of the genetic variants of OXTR on the neural activity associated with maternal behavior have yet to be revealed. In the present study, we show the genetic variants influences on the prefrontal activations measured with near-infrared spectroscopy for mothers who viewed video clips of their own child smile and other unfamiliar child smile. On the other hand, another important aspect to be noted is the phenomenon that changes in hormone levels during pregnancy to motherhood seem to make females' brain maternal. To investigate this phenomenon, we here show our preliminary studies of the developmental changes of the maternal prefrontal activations in response to children's facial expression discrimination task during pregnancy to motherhood. No potential conflicts of interest were disclosed.

(COI: No)

Symposium 45

The time in Anatomy and Physiology

(March 22, 17:30～19:00, Room G)

S45-1

Age related decline in reproductive functions and circadian rhythms

Nakamura, Wataru (*Lab. Oral Chronobiol. Grad Sch Dent, Osaka Univ, Osaka, Japan*)

In mammals, the estrus cycle is regulated by dual control of the hormone system and circadian rhythms via the hypothalamus-pituitary-gonadal axis (HPG-axis). The suprachiasmatic nucleus (SCN) is the main pacemaker of circadian rhythms, and coordinates the daily rhythms of various physiological functions and behaviors. In this study, we found that middle-aged Cry1- or Cry2-deficient female mice, which had shortened and lengthened circadian rhythms, respectively, had markedly lower pregnancy success rates compared to middle-aged wild-type (WT) mice. While young adult Cry -deficient female mice showed regular estrus cycles and normal reproductive functions from conception through to birth, middle-aged Cry-deficient female mice showed extended, irregular estrus cycles. These results suggested that the reproductive function of Cry-deficient female mice declines due to the early onset of aging-associated changes. Surprisingly, we found that if middle-aged Cry-deficient female mice with irregular estrus cycles and reduced pregnancy success rates were reared in a light/dark environment similar to their particular circadian cycle, the regularity of the estrus cycle improved and pregnancy success rates increased significantly. These results showed that the disorganization between the 24-h environmental rhythm and the circadian clock rhythm is a direct cause of the early aging-like decline in the reproductive function observed in Cry-deficient female mice.

(COI: No)

S45-2

Dynamic expression of Notch ligand Dll1 during development

Shimojo, Hiromi[1]; Isomura, Akihiro[2]; Ohtsuka, Toshiyuki[2]; Miyachi, Hitoshi[2]; Kageyama, Ryoichiro[1,2] ([1]*iCeMS, Kyoto Univ, Kyoto, Japan;* [2]*Inst. for Virus Research, Kyoto Univ, Kyoto, Japan*)

Cell-cell communications play an important role in cell fate determination. Notch signal is transmitted by cell-cell interactions and regulates formation of various tissues. Upon activation of Notch signaling, Notch effector gene Hes1 is activated and represses the expression of Dll1. It generates heterogeneous cell populations by lateral inhibition during neural development, whereas it forms homogeneous cell populations by synchronization during somitogenesis. However, how Notch signaling produces such opposite outcomes remains unclear. We previously found that the expression of Notch signal components oscillate with a period of 2-3 hr in neural progenitors. These results suggest that the regulation of Notch signaling is more dynamic than previous thought. To reveal the significance of Notch signal dynamics in various developmental events, we visualize the expression of Dll1 protein during neural development and somitogenesis. Live-cell imaging revealed that the expression of Dll1 protein oscillates in phase between neighboring cells during somitogenesis. By contrast it oscillates out of phase between neighbors during neural development. Cell-cell interactions dynamically change in both events, but the dynamics between neighboring cell is differently controlled, depending on tissue types. These results suggest that dynamics of Notch signaling regulate the formation of homogeneous and heterogeneous cell population by controlling of phase of Notch signal oscillation between neighboring cells.

(COI: No)

S45-3

Inhibitory maturation regulates the critical period plasticity for binocular vision

Sugiyama, Sayaka (*Lab of Neuronal Dev., Grad School of Med. Dent. Sci., Niigata Univ. Niigata, Japan*)

Binocular vision is established in the primary visual cortex (V1) through activity-dependent competition in early postnatal life. During the critical period, monocular deprivation (MD) yields a strong shift in cortical responsiveness toward non-deprived eye concomitant with a rapid pruning of dendritic spines and later axonal remodeling, hence causes a permanent deficit in deprived-eye vision (amblyopia). Growing evidence demonstrates that distinct GABAergic circuits drive the critical period plasticity for binocular vision. Transfer of Otx2 homeoprotein into parvalbumin (PV)-cells activates this sensitive period in the visual cortex. Here, we show that genetic cascade induced by Otx2 underlies maturation of PV-cell circuits. Otx2 deletion results in reduction of mature PV-cells enwrapped by chondroitin sulfate (CS) proteoglycans. Our data shows that CS reduction by genetic deletion of one CS enzyme causes the impairment of PV-cell function and disruption of plasticity. Similarly, Otx2 induces specific expression of actin-remodeling factor for plasticity. Thus, once PV-cell internalizes Otx2, extracellular and intracellular machineries activated by this homeoprotein may cooperate together in maturation of PV networks hence the cortical plasticity.

(COI: No)

S45-4

The neural mechanism of vocal signal transmission beyond generations in songbirds

Abe, Kentaro[1,2] ([1]*Grad Sch Med, Kyoto Univ, Kyoto, Japan;* [2]*JST, PRESTO, Kawaguchi, Japan*)

During the evolutional history of life, organisms continuously replicate themselves through reproductive processes. Through such repetitive cycle of generation, an organism pass the information on to the next generation as an inherited genome. In this respect, modern humans are peculiar in their features that they can also accumulate and pass on non-genetic information beyond generations through social interactions. To study the biological basis of neural mechanisms that allow such kind of non-genetic passage of information, I have studied the individual development of the skills to vocally communicate with other in songbirds. Similarly to the acquisition process of language skills in humans, proper postnatal social experiences largely influence, and are critically necessary for, the acquisition of such skills in songbirds. Using a specific postnatal education paradigm concomitantly with transgenic technology in songbirds, we separately manipulate both intrinsic and extrinsic factors involved in the development of vocal skills. Taking this approach, we investigated the molecular mechanism of how social interaction stimulates the acquisition of postnatally learned behaviors, i.e., vocal performance. We found that efficient skill acquisition is executed by coordinating such intrinsic information and extrinsic information, and a transcription factor which shows a neural activity dependent gene transcription activity play an important role in this process. Our results provide an insight into the broader question of how social influences affect the postnatal acquisition of behaviors or inherited information.

(COI: No)

S45-5

Regulatory mechanism of biological rhythm and instinctive behaviors

Yamanaka, Akihiro (*Dept Neuroscience II, Inst Env Med, Nagoya Univ, Nagoya, Japan*)

Neurons form complex network which work as functional circuit to regulate behavior in the brain. Little is known about how these circuit functions to regulate behavior since it was impossible to control the activity of specific type of neurons among them. Recently developed techniques, optogenetics and pharmacogenetics (chemicogenetics) enables control the activity of specific type of neurons in the brain using light or chemical substances. These new techniques allow us to study the function of these network and behavior using the whole animal. Especially, instinctive behaviors such as feeding, drinking and sleep/wakefulness behaviors are exhibited only in the whole animal. To reveal its regulatory mechanism, in vivo study using whole animal is essential. I developed a series of transgenic mice line which allow us to easily apply optogenetics. These instinctive behaviors are regulated by neuropeptide containing neurons in the hypothalamus. The activity of these peptidergic neurons was acutely manipulated using optogenetics or pharmacogenetics to control instinctive behaviors. In this symposium, I will discuss about neural regulatory mechanism of these instinctive behaviors using our recent results.

(COI: No)

Symposium 46

Structure and function of the hippocampus: approach from molecule to neuronal network

(March 22, 17:30～19:00, Room H)

S46-1

Conversion mechanism of temporal Ca^{2+} code into persistent biochemical code during LTP

Hayashi, Yasunori (*RIKEN BSI*)

Upon induction of long-term potentiation (LTP), a transient Ca2+ signal through NMDAR is converted into a long-lasting increase in synaptic transmission and an enlargement of the dendritic spines. There must be a point within the signaling cascade where transient Ca2+ code is converted to a persistent biochemical code. Here, we identified a formation of a stable heterooligomer between CaMKII and TIAM1, a Rac specific guanine-nucleotide exchange factor (GEF), as the point of the conversion. The binding of TIAM1 on "T-site" of CaMKII prevents autoinhibition and locks CaMKII in an active conformation similarly to the action of T286 autophosphorylation, which, in turn, results in a persistent activation of TIAM1. This mutually activating complex persistently activate of Rac, which is required for the maintenance of sLTP. TIAM1/CaMKII can form a ternary complex with NR2B, through the dodecameric structure of CaMKII, thereby maintaining the active complex at the vicinity of NMDAR. In this way, CaMKII acts as a structural and signaling hub that, once activated by Ca2+, can persistently activating TIAM1 and possibly other the signal transduction molecules at the vicinity of the activated synapse.

(COI: Properly Declared)

S46-2

Large-volume analyses of synapse nanostructure by automated section-collecting system

Iwasaki, Hirohide[1,2]; Isshiki, Masaaki[1]; Ito, Aya Ishida[1]; Kashiwagi, Yutaro[1,2]; Okabe, Shigeo[1,2] ([1]*Grad.Sch.Med.Univ.Tokyo., , Tokyo, Japan;* [2]*CREST, JST*)

A huge number of neurons in the brain are connected each other via synapses and form functional circuits. There are three prevailed models of synaptogenesis, Miller-Peters model, Sotelo model and filopodia model. However, our recent imaging studies indicate the presence of other types of mechanisms that support synaptogenesis in either cortical interneurons or cerebellar parallel fibers. These observations suggest the importance of comprehensive structural analyses of synapse formation. Structural data of synapses can be obtained by either light microscopy or electron microscopy. Although light microscopic detection of spiny protrusions and clustering of postsynaptic molecules has been utilized as markers of excitatory postsynaptic sites, structural features of spine synapses at a nanometer-scale resolution can only be achieved by electron microscopic observation. Conventionally, structures of spine synapses are analyzed by the three-dimensional (3D) reconstruction of dendrites after transmission electron microscopy. However, 3D reconstruction of dendrites is a labor-intensive procedure and the image reconstruction is inevitably limited to a small volume. In order to obtain comprehensive data for the 3D morphology of spine synapses in a large tissue volume, new technologies are required. An approach that combines both light and electron microscopy by using automated section-collecting system, ATUM, is a possible solution for the comprehensive analyses of synapse nanostructure. The technical details of this correlative microscopic approach will be presented.

(COI: No)

S46-3

Hippocampal EEG dynamics of virtually locomoting mice

Katayama, Norihiro (*Biomodeling Lab, Grd Schl Info Sci, Tohoku Univ, Japan*)

The hippocampus plays important role in spatial recognition and navigation. There are many studies reporting that the rhythmic EEG at 6-12 Hz (theta rhythm) occurred in the hippocampus. The theta activity is strongly correlated with the speed of locomotion of the animal. In addition, the theta activity is modulated by several sensations such as vestibular sensation. However, contribution of visual feedback to the theta activity has not been well disclosed. In this study, we investigated the relationship between the locomotion speed and the hippocampal theta rhythms of mice freely behaving in a virtual environment under head-restrained condition. We would like to show some data suggesting the contribution of sensory modalities to the hippocampal theta EEG activities.

(COI: No)

S46-4

Recent advances in anatomical research on the perineuronal net in the hippocampus

Jinno, Shozo (*Dept Dev Mol Anat, Grad Sch Med Sci, Kyushu Univ, Fukuoka, Japan*)

Recent studies have suggested that the perineuronal net (PNN), a specialized extracellular matrix structure, and parvalbumin (PV), an EF-hand calcium-binding protein, are involved in the regulation of neural plasticity. Here, we aimed to quantitatively estimate the relationship between the two plasticity regulators, PV and PNN, in the hippocampus of young adult mice. Dual fluorescence staining for PV and *Wisteria floribunda* agglutinin (WFA; a broad PNN marker) showed that a substantial population of PV-positive (PV+) GABAergic neurons were PNN-negative (PNN-). Optical disector analysis demonstrated that there were fewer PNN+ neurons than PV+ neurons. The ratio of PNN expression in PV+ neurons was generally lower in the dendritic layers than in the principal cell layers, while the ratio of PV expression in PNN+ neurons was effectively 100%. The mean PV fluorescence was significantly higher in PNN+/PV+ neurons than in PNN-/PV+ neurons. Cumulative frequencies for single-cell PV fluorescence indicated that intensely stained PV+ neurons tend to be enwrapped by PNNs, while weakly stained PV+ neurons are likely to lack PNNs. We digested the PNNs by a unilateral injection of chondroitinase ABC (chABC) into the dorsal CA1 region. Although the densities of PV+ neurons remained unchanged, the PV fluorescence declined 7 days after chABC injection. Quantitative real-time polymerase chain reaction analysis demonstrated a reduction in PV mRNA expression following chABC injection. These findings indicate that the presence or absence of PNN affects the relative PV expression in GABAergic neurons in the hippocampus.

(COI: No)

S46-5

Local control of axonal excitability of hippocampal mossy fibers

Kamiya, Haruyuki (*Dept Neurobiol, Grad Sch Med, Hokkaido Univ, Sapporo, Japan*)

Axons are the sole outputs of the neurons, and carry neuronal information reliably to the target cells. Although axonal excitability has been shown to be modulated by subtle changes in the local micro-environment, detailed mechanisms and consequences of local control of axonal excitability were rarely tested. It has been demonstrated that bath application of low concentration of kainate (an agonist of kainate receptors) or muscimol (an agonist of $GABA_A$ receptors) enhances the excitability of hippocampal mossy fibers. In this study, we attempted to localize the sites of actions of kainate and muscimol. For this purpose, we adopted a quantitative focal application of these agonists to the distal axons of the mossy fibers. Unexpectedly from digital nature of propagation of action potentials along the axons, the size of presynaptic fiber volleys, the compound action potentials recorded extracellularly, increased by local application of low concentration of kainate to the distal axons of mossy fibers. These effects might reflect mild depolarization of either presynaptic or postsynaptic membranes, or both, since application of the solution containing a slightly higher concentration of potassium ions showed similar effects. Application of muscimol also enhanced presynaptic fiber volleys due to the excitatory effect of presynaptic $GABA_A$ receptors, possibly by the higher intracellular concentration of chloride ions within mossy fiber axons. We also carried out computer simulations of propagation of action potentials along the realistic model of mossy fibers. The mechanisms underlying modulation of presynaptic fiber volleys will be discussed.

(COI: No)

Symposium 47

New streams in researches knitted with neurophysiology and stem cell histology

(March 22, 17:30～19:00, Room I)

S47-1

Cell cycle analysis of endogenous neural precursors in the adult mouse brain after brief seizures

Mori, Tetsuji[1,2]; Wakabayashi, Taketoshi[2]; Hirahara, Yukie[2]; Takamori, Yasuharu[2]; Koike, Taro[2]; Kurokawa, Kiyoshi[2]; Yamada, Hisao[2] (*[1]Sch. Med. Tottori Univ., Yonago, Japan; [2]Kansai Medical Univ., Hirakata, Japan*)

Endogenous neural precursors reside in the subvendymal zone (SVZ) lining the lateral ventricle and the subgranular zone (SGZ) of the hippocampus throughout the life of mammalian. Many studies show that neurogenesis in these regions is enhanced by various stimuli, such as stroke and status epilepticus. Although there are many studies about the responses of SGZ precursors to epileptic seizures, few have examined effects on SVZ precursors. In this study, we focused on the responses of adult SVZ precursors to brief generalized clonic seizures induced by a single administration of pentylenetetrazole (PTZ), a commonly used chemoconvulsant. PTZ-induced brief seizures are much milder than status epilepticus, eliciting no obvious neuronal cell death. We found that brief seizures immediately resulted in cell cycle inhibition of SVZ precursors. This initial inhibition was followed by reduced cell cycle length and enhanced cell cycle re-entry after the first round of mitosis (20 hours after PTZ administration), leading to precursor pool expansion. However, the expansion of the precursor pool was transient. On the other hand, SGZ precursors showed different responses to the PTZ-induced seizures. The precursor pool of the SGZ transiently expanded three days after PTZ administration without obvious cell cycle inhibition. These results suggest that adult neurogenesis is susceptible to excessive neuronal excitation, and neurogenesis in the adult SVZ is more tightly regulated than that in the adult SGZ.

(COI: No)

S47-2

Neuronal migration for maintenance and repair of adult brain

Sawamoto, Kazunobu (*Grad.Sch.Med.Sci.Nagoya City Univ., Nagoya, Japan*)

Neuronal migration is an important process in brain development and homeostasis. It is not only a phenomenon of embryogenesis: it also occurs in the adult brain, following adult neurogenesis. In fact, throughout life, numerous new neurons generated by stem cells in the adult ventricular-subventricular zone (V-SVZ) take the long journey (millimeters to centimeters, depending on the species) to the olfactory bulb (OB) through the rostral migratory stream (RMS). In the adult rodent V-SVZ and RMS, new neurons migrate in chains through astrocytic tunnels. After reaching the OB, the new neurons migrate radially and decrease their speed to stop in their final positions. New neurons are recruited into the empty position generated by cell death and the sensory input promotes the reiterated use of the same positions by new neurons. This mechanism may contribute to the stability and plasticity of the adult brain during neuronal turnover. The neural stem cells in the adult V-SVZ also have the capacity to partially regenerate new neurons after various insults. After ischemic injury in rodents, the V-SVZ-derived new neurons migrate from the V-SVZ towards the injured site along blood vessels. In this talk, I will present recent studies on the mechanisms of neuronal migration occurring in the adult brain of various animals under physiological and pathological conditions.

(COI: No)

S47-3

NG2-expressing progenitor cells maintain neuronal function by controlling local environment in the central nervous system

Kataoka, Yosky[1,2]; Nakano, Masayuki[1,2]; Yamato, Masanori[1]; Tamura, Yasuhisa[1] (*[1]Cellular Function Imaging Team, RIKEN Center for Life Science Technologies, Kobe, Japan; [2]Dept Physiol, Grad Sch Med, Osaka City Univ, Osaka, Japan*)

Progenitor cells expressing chondroitin sulfate proteoglycan 4 (NG2-expressing progenitor cells) are ubiquitously distributed throughout the gray and white matter in the central nervous system of adult mammals. NG2-expressing progenitor cells have been known to show the proliferative activity and give rise to mature oligodendrocytes. We reported that the cell fate of progenitor cells is shifted from oligodendrocytes to astrocytes depending on depolarizing stimuli to the brain, indicating that the progenitor cells are involved in activity-dependent tissue remodeling. We here discuss a new concept that NG2-expressing progenitor cells maintain neuronal function by controlling the local immune response. The progenitor cells are known to receive direct synaptic inputs from neurons, and are often located adjacent to neuronal somata, suggesting functional interaction between the progenitor cells and neurons. Recently, we succeeded in rapid and selective ablation of NG2-expressing progenitor cells. The ablation showed neuronal cell death in the hippocampus with increased expression of pro-inflammatory cytokines and pro-apoptotic genes. In the animals, hippocampal neurons contained death receptors involved in the signaling pathway for apoptosis. These observations suggest that NG2-expressing progenitor cells maintain neuronal survival by regulating local environment including the immune system.

(COI: No)

S47-4

Morphologies and Functions of Olig2-positive cells in the adult brain

Wanaka, Akio; Tatsumi, Kouko; Okuda, Hiroaki; Morita, Shoko (*Sch. Med. Nara Med. U., Nara, Japan*)

Olig2 is a member of a basic helix-loop-helix transcription factor family and regulates differentiation of motor neurons and oligodendrocytes in the embryonic neural tube. Olig2-positive cells often co-express NG2-proteoglycan and persist to adulthood. The Olig2/NG2 cells in the adult brain are regarded as oligodendrocyte precursor cells (OPCs), which have the potential to differentiate into oligodendrocytes, astrocytes or neurons. We employed a double transgenic mouse that can mark the Olig2-positive cells with membrane-targeted EGFP. The genetic labeling revealed that Olig2-positive cells preferentially differentiated into astrocytes in the mechanically injured cerebral cortex, while they showed oligodendrocytic differentiation in the demyelinated corpus callosum of cuprizone-fed mice. During these genetic labeling experiments, we noticed that Olig2-positive cells did not always co-express NG2 proteoglycan. Especially, in the basal ganglionic nuclei, Olig2-single positive cells were predominant and positive for GFAP immunoreactivity. We allowed the double transgenic mice run voluntarily for three weeks and compared the morphological characteristics of the Olig2-positive cells before and after voluntary running in the Globus pallidus. The Olig2-positive astrocytes transformed to bushy astrocytes by elaborating their fine processes after three-week running. These findings suggested that Olig2-positive cells in the adult brain changed their morphologies in response to pathological and physiological conditions.

(COI: No)

S47-5

A role of immune cells on brain repair

Matsuyama, Tomohiro; Nakagomi, Takayuki; Doi, Akiko; Kawahara, Maiko; Sakuma, Rika (*Lab Neurogenesis, Inst Adv Med Sci, Hyogo Col Med, Hyogo, Japan*)

We have found a new type of endogenous neural stem cells induced by cerebral ischemia (Eur J Neurosci 2009;29:1842). These cells are derived from vascular pericytes and express hematopoietic markers (Stem Cells Dev 2011;20:2037). Brain pericytes are a key component of neurovascular unit. Here, using the ischemic pericytes (iPC) of mouse brain and the human brain pericytes cultured under oxygen glucose deprivation, we show that pericytes developed the stemness through reprogramming. The iPC revealed a complex phenotype of neuroangioblast in addition to mesenchymal properties and differentiated into neural and vascular lineage cells. These data indicate that under ischemia pericytes can be reprogrammed into multipotent stem cells to differentiate into all components of neurovascular unit, suggesting that iPC contribute to both neurogenesis and vasculogenesis. However, such reparative mechanisms are often disturbed by immune response in injured brain. We already have demonstrated that CD4 T cells serve as negative modulators in neurogenesis after stroke (J Neurosci Res 2010;88:2385). Glucocorticoid-induced tumor necrosis factor receptor (GITR), a TNF receptor superfamily expressed on activated CD4 T cells, has a key role on brain repair after stroke (Cell Death Differ 2012;19:756). GITR triggering on CD4 T cells increases brain inflammation and decreases the iPC. These observations indicate that activated T cells are major deteriorating modulators of both neurogenesis and angiogenesis. This suggests that blockade of the GITR interaction may be a novel immune based therapy in stroke.

(COI: No)

Symposium 48

New structural and functional logics governing electrical signal propagation

(March 22, 17:30～19:00, Room J)

S48-1

The cerebral cortex has another neural network as its basic structure: the dendritic reticulum formed by gap junctions

Fukuda, Takaichi (*Grad.Sch.Med.Kumamoto Univ., Kumamoto, Japan*)

A subpopulation of cortical GABAergic neurons that contain parvalbumin (PV) use not only chemical synapses but also gap junctions for their communication. Gap junctions are formed between dendrites and allow direct transmission of electrical signals with minimal delay, thus they are thought to facilitate synchronous neuronal activities. Though some technical reasons make it difficult to detect neuronal gap junctions in the brain, we have recently developed a method to overcome the difficulty. Analysis in the visual cortex has shown that the dendritic linkage was not that of a simple cell-to-cell connection but constituted the reticulum formed by multiple dendrites that came close together. Reconstruction of coupled neurons revealed the three linkage types. First, PV neurons in close proximity formed mutual connections through proximally located gap junctions. Second, vertical dendrites bridged somata located at some distance inside the same columnar space. The third type was observed between PV neurons located in neighboring columns. Importantly, in both type 2 and 3 linkages, at least one of the two somata was located within the distance less than 100 μm from the connecting gap junction. This arrangement and previous physiological data suggest a role of distant gap junctions that they transmit action potentials as depolarizing synchronous signals between inhibitory neurons in one direction, whereas gap junctions among clustered neurons might mediate bidirectional synchronous signals. These structures may drastically update our knowledge on organizing principles in the cortical circuitry.

(COI: No)

S48-2

Microglia and synapse interactions: microglial contribution for synapse formation during development

Miyamoto, Akiko[1]; Wake, Hiroaki[1,2]; Murakoshi, Hideji[3]; Eto, Kei[1]; Nabekura, Junichi[1,2] ([1]*Dept Homeostatic Develop, Natl Inst Physiol Sci, Aichi, Japam;* [2]*Dept Physiol, Sch Life Sci, SOKENDAI, Kanagawa, Japapn;* [3]*Supportive Center for Brain Research, Natl Inst Physiol Sci, Aichi, Japan*)

Microglia, which are the immune cells in the central nervous systems, are one of the glial cells. Because of this immune cell character, microglial functions at injured or pathological condition have been well studied. Over the last decade, it has been revealed that microglia also have some actions for synaptic function and connection during physiological condition using imaging and electrophysiological techniques. For example, microglia selectively contact onto synapses in intact brain and are also involved in circuit refinement via synapse elimination at ischemic penumbra region and developmental period, which may contribute to neural circuit reorganization. Recently, we also found that microglia induce filopodia which are precursor of spine during cortical development using in vivo two photon imaging technique. We observed that filopodia was formed at microglial contacted dendrite of L2/3 pyramidal cell. Injection of microglia activation inhibitor or their ablation induced by genetic manipulation decreased cortical spines in density. We also examined miniature EPSC frequency and it was significantly reduced in microglia ablated mice. Taken together, This finding suggests that microglia contributed to neuronal circuit maturation not only via synapse elimination but also via synapse formation during development.

(COI: No)

S48-3

A novel stromal cell network visualized by FIB/SEM tomography

Nakamura, Keiichiro[1]; Hagashi, Ryuhei[1]; Nguyen, Michael[2]; Lang, Richard[2]; Hirashima, Shingo[1]; Kanazawa, Tomonoshin[1]; Takeya, Mitsue[1]; Hayashi, Tokumasa[1]; Hashitani, Hikaru[3]; Ohta, Keisuke[1] ([1]*Kurume Univ. Sch.Med., Fukuoka, Japan;* [2]*Monash Univ., Melbourne, Australia;* [3]*Nagoya City Univ., Nagoya, Japan*)

Fibroblasts or stromal cells are a dominant cell type in the connective tissue of various organs, and are connected to one another by gap junctions to form cellular networks. However, it is not easy to understand the whole structure of those cells or their spatial relationships by light microscopy because of thinness of their processes, nor by electron microscopy as these processes extend well beyond a single thin section. The recent development of a novel electron microscopic technology, FIB/SEM tomography, enables us to establish 3D ultrastructure of these cells. Using FIB/SEM, we can obtain over 1000 serial images, these images are then aligned and the 3D cell structures reconstructed using computerized segmentation instrumentation. In the present study, we concentrated on visualizing stromal cells in the renal pelvic and seminal vesicle. We showed in both organs that stromal cells or fibroblasts, which are recognized by their long thin cytoplasmic processes in a single section, are cells with very broad, thin and wavy "sheet-like" cytoplasmic processes. They approach one another at some points along their edge to form an incomplete wall between the epithelial layer and connective tissue beneath it. The stromal cells in the muscular layer of the seminal vesicle form a honeycomb-like structure which encircle the smooth muscle bundles. Thus these structures seem to create spatial divisions to form functional units within tissues.

(COI: No)

S48-4

Ca^{2+}- and voltage-dependent activation of TRPM4 channel may account for abnormal automaticity

Inoue, Ryuji[1]; Hu, Yaopeng[1]; Zhu, Xin[2]; Numata, Tomohiro[1] ([1]*Dept Physiol, Grad Sch Med, Fukuoka Univ, Fukuoka, Japan;* [2]*Biomed. Info. Tech. Lab. the Univ. Aizu, Fukushima, Japan*)

In the heart, under some pathological conditions, abnormal automaticity, which emerges as ectopic and repetitive spontaneous depolarizations, often leads to tachyarrhythmias. In this study, we performed a simultaneous recording of membrane potential and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), and analyzed the antiarrhythmic effects of a TRPM4 channel blocker 9-Phenanthrol (9-PA) on spontaneous action potentials (APs) in cultured HL-1 atrial myocyte clusters. In the majority of spontaneously beating HL-1 clusters, the rate of AP firing was irregular, but in a small number of the clusters, a regular and slower AP firing pattern reflecting the pace-making activity of I_f current was observed. Both regular and irregular APs were synchronized with transient elevations of $[Ca^{2+}]_i$ and strongly accelerated by isoproterenol (Iso; 0.1 μM) or $BaCl_2$ (0.1mM) which reportedly inhibits an inward-rectifying K^+ current in HL-1 myocyte, with depolarization of resting membrane potential and increased basal $[Ca^{2+}]_i$ level. These changes were abolished by 9-PA (10 μM). Moreover, in myocytes depolarized by $BaCl_2$, 9-PA decreased the slope of pre-AP depolarization, diminishing the AP firing rate. Mathematical simulations based on an HL-1 AP model indicated that Ca^{2+}- and voltage-dependent activation of TRPM4 is responsible for Iso-induced depolarization and concomitant increase in AP firing. This mechanism might contribute to the pathogenesis of tachyarrhythmias such as non-reentrant atrial tachycardia.

(COI: No)

Symposium 49

Regulation of physiological functions by neuroactive steroid and its morphological foundations: Regulatory mechanism for GABA signaling

(March 23, 9:00～10:30, Room D)

S49-1

Morphological appearance of GABAergic neuroactive steroid-synthesizing enzymes

Tsuruo, Yoshihiro (*Dept Anat Cell Biol, Inst Health Biosci, Univ Tokushima Grad Sch*)

Neuroactive steroids are synthesized in neural tissues as well as peripheral endocrine organs. They have a variety of neuromodulatory actions by the interactions of classical nuclear receptors and also by the allosteric modulation of γ-aminobutyric acid type A ($GABA_A$) receptors. The neuroactive steroids, allopregnanolone (3α-hydroxy-5α-pregnan-20-one) and tetrahydrodeoxycorticosterone (3α, 21-dihydroxy-5α-pregnan-20-one) are endogenous potent positive allosteric modulators of $GABA_A$ receptor function, and they are involved in anxiolytic , sedative, analgesic and anticonvulsant actions by opening the GABA-gated chloride channel. These neuroactive steroids are synthesized from progesterone and deoxycorticosterone, respectively. The sequential catalysis of metabolism is mediated by the two enzymes: 5α-reductase (5α-R) and 3α-hydroxysteroid dehydrogenase (3α-HSD). Two isozymes of 5α-R are found in rodents and human, and 3α-HSD has four isoforms in human and only one type in rodents. The expression of 5α-R or 3α-HSD is shown immunohistochemically using the specific antibodies against these enzymes in rodent neural tissues as well as peripheral endocrine organs.

(COI: No)

S49-2

GABAergic signaling in the developing CNS-GABAergic neurons and $GABA_A$ receptors-

Takayama, Chitoshi; Shimizu, Chigusa (*Sch. Med., Univ of Ryukyu, Okinawa, Japan*)

In the adult central nervous system (CNS), gamma-amino butyric acid (GABA) is a predominant neurotransmitter. GABA is synthesized by glutamic acid decarboxylase (GAD), and packed into synaptic vesicles by vesicular GABA transporter (VGAT) in the axons terminals of GABAergic neurons. After released from presynaptic terminals by exocitosis, GABA binds to the $GABA_A$ receptors ($GABA_AR$) on the postsynaptic membrane, induces hyperpolarization of membrane potential, and is rapidly transported into the presynaptic terminals and glial processes surrounding the synapses by the GABA transporters (GAT). We have been investigating the development of these GABAergic systems in various regions in the CNS, such as cerebellum, cerebral cortex and spinal cord, by immunohistochemistry for GABA, GAD, VGAT, GABAAR , GATs. Commonly, GABAergic neurons appeared far before the synapse formation, and GABA may be released by non-exocitotic system. Before synapse formation, extrasynaptically released GABA binds immature types of $GABA_AR$, mediates depolarization of membrane potential, since intracellular Cl- concentration is low due to the lower expression of K^+, Cl^-, co-transporter2 (KCC2). Furthermore, the depolarization activates voltage dependent calcium channel, induces Ca^{2+} influx, and the excitatory action of GABA might be involved in morphogenesis of CNS. After synapses are formed, released GABA binds the mature types of $GABA_AR$, and is involved in inhibition of glutamatergic activity. In this symposium, we demonstrate the changes in the GABAergic system in the cerebellum and cerebral cortex.

(COI: No)

S49-3

Neurosteroid actions on GABA-A receptors: sites and mechanisms

Steinbach, Joe-Henry (*Dep Anesthesiol, Sch Med, Washington Univ, St Louis, USA*)

Potentiating neurosteroids increase the strength of neuronal inhibition by enhancing the activity of GABA-A receptors, increasing the response to lower concentrations of GABA and prolonging the synaptic current. Potentiation occurs because neurosteroids reduce the rate at which the open channel closes, while the affinity of the receptor for the neurotransmitter GABA is not changed. Potentiating steroids also enhance the response to allosteric activators of the GABA-A receptor that can open the channel but bind to sites that are different from the GABA-binding site. Overall, the basic mechanism is to affect receptor kinetics rather than receptor affinity for agonists.Potentiating steroids interact with the 1st membrane-spanning region (TM1) of the α subunit. When a single residue in this region is mutated potentiation by steroids (but not other drugs) can be removed. However, potentiation can be restored to the receptor by mutations that convert the TM1 of the β or γ subunit to the sequence in α. This observation indicates that it is not a unique property of the α subunit, or of the subunits adjacent to the α subunit that allows potentiation. The native α subunit is involved in binding both steroid and GABA itself. However, potentiation does not require that a single subunit bind both drugs since receptors can be constructed that lack one or the other site on selected subunits, and potentiation is preserved. These findings indicate that steroid binding affects the gating properties of the receptor as a whole, rather than changing the function of a single subunit.

(COI: No)

S49-4

Role of neuroactive steroids in adrenal medullary cells

Inoue, Masumi; Harada, Keita; Matsuoka, Hidetada (*Dept Cell and Systems Physiol, Sch Med, Univ Environ and Occup Health, Kitakyushu, Japan*)

GABA is present not only in the central nervous system, but also in peripheral tissues, and exerts a variety of physiological functions via two kinds of receptors, ionotropic GABAARs and metabotropic GABABRs. In the adrenal gland, one of GABA-synthesizing enzymes, GAD67, is expressed in adrenal medullary (AM) cells and GABA is stored in chromaffin granules, but not in synaptic-like microvesicles. GABAARs consisting of α3, β, and γ2 subunits are mainly expressed in AM cells, whereas the expression of GABA transporters (GATs), which are involved in the clearance of GABA at GABAergic synapses, are absent in the adrenal medulla. GABA induces a depolarization with the consequent enhancement of catecholamine secretion. The GABAAR function in AM cells is increased by allopregnanolone, a neuroactive steroid which is produced in the adrenal cortex. Allopregnanolone induces a leftward shift of the dose-response curve for GABA. The EC50 of GABA in the presence of 0.1 μM allopregnanolone decreases to less than one-tenth of that in the absence. Furthermore, the expression of α 3 subunits is enhanced by glucocorticoids. These results indicate that adrenal steroids play an important role for the regulation of GABA-mediated functions in AM cells. (CO1:No)

(COI: No)

Symposium 50

Frontier on fatigue, autonomic nerve dysfunction, and sleep-rhythm disorder

(March 23, 9:00～10:30, Room F)

S50-1

Fatigue and its correlates of autonomic nervous system, sleep, and circadian rhythm disorders

Tanaka, Masaaki[1]; Ishii, Akira[1]; Watanabe, Yasuyoshi[1,2] (*[1]Dept Physiol, Osaka City Univ Grad Sch Med, Osaka, Japan; [2]RIKEN, Center for Life Science Technologies, Hyogo, Japan*)

Fatigue is defined as a condition or phenomenon of declined ability and efficiency of mental and/or physical activities, caused by excessive mental or physical activities, diseases, or syndromes; it is often accompanied by a peculiar sense of discomfort, a desire to rest, and reduced motivation, referred to as fatigue sensation. Acute fatigue is a normal condition or phenomenon that disappears after a period of rest; in contrast, chronic fatigue, lasting at least 6 months, does not disappear after ordinary rest. Chronic fatigue impairs activities and contributes to various medical conditions, such as cardiovascular diseases, epileptic seizures, and death. In addition, many people complain of chronic fatigue. For example, in Japan, more than one third of the general adult population complains of chronic fatigue. It would thus be of great value to clarify the mechanisms underlying chronic fatigue and to develop efficient treatment methods to overcome it. In this symposium, we would like to review data primarily from behavioral, electrophysiological, and neuroimaging experiments related to neural dysfunction as well as autonomic nervous system, sleep, and circadian rhythm disorders in fatigue. These data provide new perspectives on the mechanisms underlying chronic fatigue and on overcoming it.

(COI: No)

S50-2

Autonomic nerve alteration caused by fatigue in children and adolescents

Mizuno, Kei[1,2]; Joudoi, Takako[4] (*[1]Pathophysiol Health Sci, RIKEN Cent Life Sci Technol, Kobe, Japan; [2]Dept Med Sci Fatigue, Osaka City Univ Grad Med, Osaka, Japan; [3]Osaka City Univ, Cent Health Sci Innov, Osaka, Japan; [4]Dept Pediatrics, Kumamoto Univ Hosp, Kumamoto, Japan*)

atigue induces an alteration of autonomic nerve function. An enhancement of sympathetic nerve activity based on a decrease in parasympathetic nerve activity measured by electrocardiogram (ECG) and accelerated plethysmography (APG) is closely associated with fatigue in children and adolescents. In addition to the ECG and APG, we found that skin conductance response (SCR), which is an index of sympathetic nerve activity, is also sensitive for detecting the abnormality of autonomic nerve function in patients with childhood chronic fatigue syndrome (CCFS). These results suggest that autonomic nerve alteration is a physiological marker for fatigue severity and the intervention effect on recovery from fatigue. By using the SCR and plethysmography, we are now trying to evaluate their fatigability and ability of recover from fatigue by measuring the time series variation of autonomic nerve activity for comparatively prolonged time including resting and cognitive challenge conditions. In addition, we are focusing particularly on a stimulation of the parasympathetic nerve activity and performing an intervention study to alleviate fatigue is also performing in fatigued children and adolescents. To overcome pubertal chronic fatigue, multifaceted intervention studies in relation to lifestyle modification, environmental space, and food and drug are needed.

(COI: No)

S50-3

A role of sleep and circadian rhythm in the fatigue recovery

Tajima, Seiki (*Hyogo children's sleep and development medical research center, Kobe, Japan*)

Fatigue is an indispensable bio-alarm to avoid exhaustive state caused by overwork or stresses. Sleep is well known as one of the important factors to recover fatigue. Here we show the relationships between fatigue pathology and sleep disorders. Sleep deprivation has high impact on fatigue pathology, especially in childhood. Miike et al reported that accumulation of sleep deprivation during childhood caused biological clock desynchronization. The desynchronization was expressed as circadian rhythm disorders. Under those conditions, not only clock gene expression but also endocrine rhythm, energy metabolism, cognitive function and autonomic activity were disordered. Miike also reported pediatric chronic fatigue status was prevented by just thirty minutes increase in total sleep time.On the other hand, prolonged total sleep time, increased locomotion during sleep and sleep fragmentation were essential in adult chronic fatigue state. From the viewpoint of autonomic function, vagal tone suppression was related with chronic fatigue.Those findings show that regular sleep habit is important for recover from fatigue state, and also, autonomic dysfunction leads to insufficient fatigue recovery.

(COI: No)

S50-4

Neural mechanisms of fatigue

Ishii, Akira[1]; Tanaka, Masaaki[1]; Yamano, Emi[1]; Watanabe, Yasuyoshi[1,2] (*[1]Dept Physiol, Grad Sch Med, Osaka City Univ, Osaka, Japan; [2]RIKEN Center for Life Science Technologies*)

Fatigue is a common problem in modern societies. In Japan, more than half of the adult population reports experiencing fatigue. Fatigue is defined as difficulties in initiating or sustaining voluntary activities and unpleasant sensation that accompanies fatigue (i.e., fatigue sensation) plays an important role in biological alarm to take a rest to avoid disrupting homeostasis. Over-activation of the fatigue sensation may be involved in the pathophysiology of chronic fatigue. However, the neural mechanisms of the fatigue sensation are not well-understood. We performed several neuroimaging studies to clarify the neural mechanisms of the fatigue sensation using magnetoencephalography (MEG) with high temporal and spatial resolutions. We showed that the posterior cingulate cortex (PCC) is involved in the self-evaluation of the levels of fatigue: The equivalent current dipole in the PCC and the decrease in delta band power in the PCC were observed when the levels of physical and mental fatigue, respectively, were self-evaluated. In another MEG study, the PCC and other brain regions such as the dorsolateral prefrontal cortex and frontal pole were related to the decision to rest in the presence of fatigue. In addition, we demonstrated that fatigue sensation can be classically conditioned in human and that the PCC was involved in the neural mechanisms of the classical conditioning of fatigue sensation. Our findings may help clarify the neural mechanisms of fatigue sensation and increase our understanding of the pathophysiology of chronic fatigue.

(COI: No)

Symposium 51

Multilayered physiology-anatomy joint symposium for the cerebral cortical development and maturation

(March 23, 9:00～10:30, Room H)

S51-1

Nuclear traffic of neocortical progenitor cells under the influence of mechanical factors

Miyata, Takaki (*Nagoya Univ. Grad. Sch. Med.*)

The neuroepithelium (NE) or ventricular zone (VZ), from which multiple types of brain cells arise, is pseudostratified. In the NE/VZ, neural progenitor cells are elongated along the apicobasal axis, and their nuclei assume different apicobasal positions. These nuclei move in a cell cycle-dependent manner, i.e., apicalward during G2 phase and basalward during G1 phase, a process called interkinetic nuclear migration (INM). Although INM is observed in a wide variety of epithelia, pseudostratification in the developing mammalian brain, especially in the neocortical primordium, is the most extensive and persistent during development, suggesting that neocortical NE/VZ would be a good model to study how physical or mechanical issues or parameters, such as tissue volume, cell number, and cellular traffic/flow in a given space, may affect neural progenitors' behaviors. Recent experiments in which overcrowding was induced in mouse neocortical NE/VZ, as well as comparisons of neocortical INM between mice and ferrets, have revealed that the behavior of NE/VZ cells can be affected by cellular densification. A consideration of the physical aspects in the NE/VZ and the mechanical difficulties associated with high-degree pseudostratification is important for achieving a better understanding of neocortical development and evolution.

(COI: No)

S51-2

Molecular and cellular mechanisms of corticogenesis based on the structure of radial glia

Osumi, Noriko; Kikkawa, Takako (*Dep. of Dev. Neurosci., Grad. Sch. of Med., Tohoku Univ., Sendai, Japan*)

The cerebral cortex is one of the most complex structures in the central nervous system. The primordium of the cortex consists with neural stem/progenitor cells called radial glia (RG) that are gradually proceeding proliferation and differentiation in the ventricular zone (VZ). Pax6 transcription factor is specifically expressed in the RG cells, and the expression disappears in the cortical neurons, although strong and persistent expression of Pax6 is detected in some neurons in the olfactory bulb, thalamus, amygdala, and cerebellum. Expression of Pax6 continues in adult neural stem/progenitor cells throughout life. Pax6 is weakly expressed in astrocytes, and involved in their maturation. In primates, expression of Pax6 is not only seen in RG in the VS but also in the outer radial glia (oRG) in the outer subventricular zone (OSVZ). It would be interesting to know how Pax6 functions in these primate specific oRG. Multiple and cell-type specific functions of Pax6 are governed by various downstream molecules of Pax6. These include cell adhesion molecules, markers for RG (Fabp7/BLBP and fucosyltransferase synthesizing CD15/LewisX), transcription factors (Dmrta1 and Foxp2), centrosomal proteins (ninein), and RNA-binding protein FMRP. In an evolutionary point of view, one of the important roles of Pax6 is to promote long basal processes of RG and oRG. Fabp7, a fatty acid binding protein, is actually working to make such fine processes in the rodent cortical primordium. Subcellular localization of these molecules seems to be tightly associated with mechanisms of corticogenesis.

(COI: No)

S51-3

Mechanisms of cerebral corticogenesis by migrating neurons

Nakajima, Kazunori (*Keio Univ. Sch. Med., Tokyo, Japan*)

Cortical neurons form the cortical plate (CP) in an inside-out manner, in which the late-born neurons located more superficially than the early-born neurons. Reelin, a glycoprotein secreted in the marginal zone (MZ), is crucial for this layering.
To clarify the Reelin function in vivo, we expressed Reelin ectopically in the developing cortex, and found that Reelin caused the leading processes of migrating neurons to assemble in the Reelin-rich region, which in turn induced their cell bodies to form cellular aggregates around Reelin. The late-born neurons migrated past their predecessors toward the central Reelin-rich region within the aggregates, resulting in a birthdate-dependent inside-out alignment even ectopically.
In the intermediate zone (IMZ) and CP, neurons migrate along the radial fibers (locomotion). When the leading process reaches the MZ, the soma moves rapidly towards the top of the CP, while the tip of the process remains attached to the MZ (terminal translocation). We found that the outermost region of the CP is packed with immature neurons, and named this region the primitive cortical zone (PCZ). Sequential in utero electroporation experiments suggest that the Reelin-Dab1-Crk/CrkL-C3G-Rap1-dependent switching of the migratory mode from locomotion to terminal translocation plays critical roles for the neuronal entry into the PCZ. This cascade then modulates neuronal adhesion by activating integrins, leading to the eventual birth-date-dependent layering in the neocortex. Recent analyses of the Reelin-dependent cell adhesion using mathematical modeling will also be discussed.

(COI: No)

S51-4

Epigenetic regulation of reciprocal connectivity between clonal cortical neurons

Yoshimura, Yumiko (*National Inst Physiol Sci, Okazaki, Japan*)

In the neocortex, each neuron connects to a relatively small number of neighboring neurons in a highly specific manner. Previously, it is reported that radially aligned neurons derived from the same radial glial progenitor cell preferentially establish synaptic connections in postnatal cortex. Here we show that neurons within the same layer establish cell-lineage-dependent synaptic connections, and that connection specificity is predetermined by epigenetic regulation during embryonic development. To visualize clonal neurons, we generated chimeric mice by injecting induced pluripotent stem cells marked with a fluorescent protein into wild-type mouse blastocysts. We conducted dual whole-cell recordings from presumed clonal or non-clonal neuron pairs within a layer 4 barrel in cortical slices. During postnatal development, there was a transient increase in the probability of connection for clonal neuron pairs compared with that for non-clonal neuron pairs, after which many of the connections in the clonal pairs changed from being one-way to reciprocal. This high degree of reciprocity of connections between clonal cells was abolished in clonal cells lacking DNA methyltransferase 3b (Dnmt3b), which regulates gene expression. This connection specificity was also absent in clonal neurons lacking clustered protocadherins (cPcdhs); individual neurons express various isoforms of these cell-adhesion proteins, in a Dnmt3b-dependent manner. Our findings suggest that the Dnmt3b-mediated epigenetic regulation of cPcdh expression enables clonal neurons to establish cell-lineage-specific reciprocal connections during postnatal development.

(COI: No)

S51-5

Interplay between innate circuits and neuronal activity in the formation of orientation selectivity in visual cortex

Ohki, Kenichi[1,2] ([1]*Dept Mol Physiol, Kyushu U, Fukuoka, Japan;* [2]*CREST, Japan*)

Visual functions of cortical neurons are established by activity-independent and -dependent mechanisms. A recent study reported that the progeny of single cortical progenitor cells are preferentially connected in the postnatal cortex. Here we investigated whether clonally related cells have similar preferred orientation. We found that preferred orientations of clonally related cells are similar to each other, suggesting that cell lineage is involved in the development of response selectivity in the cortex. However, not all clonally related cells share response selectivity, suggesting that cell lineage is not the only determinant of response selectivity, and later postnatal activity-dependent processes may affect the final selectivity of neurons. Here, we examined the roles of neuronal activity in the development of orientation selectivity. We used genetic silencing of cortical activity starting before the formation of orientation selectivity. Despite a strong suppression of both spontaneous and visually evoked activity throughout development, orientation selectivity of neurons in the visual cortex forms and matures normally. After the orientation selectivity matures, the distribution of the preferred orientations of neurons is reorganized. We found that this process requires spontaneous activity, but not visually evoked activity. Thus, the initial formation and maturation of orientation selectivity is largely independent of neuronal activity, and the inital selectivity is subsequently modified depending on neuronal activity.

(COI: No)

S51-6

Activity-dependent neural circuit formation in the developing cortex

Yamamoto, Nobuhiko (*Graduate School of Frontier Biosciences, Osaka University*)

How genetic and environmental factors are involved in neuronal circuit formation is one of the most intriguing issues in neuroscience. The thalamocotical (TC) projection is a suitable system to investigate this problem. Sensory TC axons form branches primarily in layer 4 of the corresponding cortical area from the onset. However, complexity of TC axon branching is known to be modified by neuronal activity including sensory-evoked and spontaneous firing. We studied the molecular mechanism of TC axon branching by molecular screening, in vitro analysis, gene transfer techniques and genetically manipulated animals. Our result shows that the netrin family member, netrin-4, promotes TC axon branching via activity-dependent expression in the developing cortex. Similarly, BDNF can also act as an activity-dependent branch-promoting factor in the later developmental stages. Sema7A also promotes TC axon branching, but its expression is at the earliest period and activity-independent. Taken together, multiple branch-regulating molecules are expressed at the different developmental stages and are likely to contribute to TC axon branching in distinct context.

(COI: No)

Symposium 52

Structure and dynamics of the motor-related neuronal circuit in brain

(March 23, 9:00～10:30, Room I)

S52-1

Precise relationship among input-output connections, somatotopic representation and zebrin stripes in the cerebellum

Sugihara, Izumi (*Dept Systems Neurophyisol, Grad Sch, Tokyo Med Dental Univ, Tokyo, Japan*)

The cerebellum is involved in adapting motor performance to sensory information that are conveyed by cerebellar input systems (mossy and climbing fiber axons). The cerebellar output system is comprised of Purkinje cell axons and neurons in the cerebellar nuclei. Morphology of single axons has suggested the projection patterns of these systems may be closely correlated with each other as well as with the zebrin stripes in the cerebellum. Nanoinjections of bidirectional tracers were made into somatotopically identified regions within the hindlimb C1 zone in copula pyramidis of the rat cerebellum. Injection sites were mapped relative to the zebrin II expression pattern and were correlated with the pattern of retrograde cell labeling within the inferior olive and the basilar pontine nuclei, and the distributions of labeled Purkinje cell terminals in the cerebellar nuclei. Zebrin bands were found to be related to both climbing fiber and mossy fiber inputs, to cortical representation of different parts of the ipsilateral hindpaw, and also to Purkinje cell terminal fields in the cerebellar nuclei. These findings indicate that there is precise topographic organization within the circuitry of input and output axons, which leads to a well-defined functional map in the cerebellar cortex and nuclei. The striped molecular expression pattern in the cerebellar cortex is tightly related with this organization. Kakenhi 25430032.

(COI: No)

S52-2

Manipulation of primate neural networks by means of modified viral vectors

Inoue, Kenichi; Takada, Masahiko (*Sys Neurosci Sec, Primate Res Inst, Kyoto Univ, Inuyama, Japan*)

Using a retrograde gene-transfer vector (NeuRet vector), we established two experimental system that permits pathway-selective cell ablation and pathway-selective gene expression, respectively. Using the former system, we attempted selective removal of the cortico-subthalamic "hyperdirect" pathway. After electrical stimulation in the motor-related areas, triphasic responses are usually detected in the internal segment of the globus pallidus (GPi). In the present study, the NeuRet vector expressing human interleukin-2 receptor α-subunit was injected into the STN of macaque monkeys. Then, immunotoxin injections were made into the SMA. In these monkeys, single neuron activity in the GPi was recorded in response to the SMA stimulation. We found that the early excitation was largely reduced with neither the inhibition nor the late excitation affected. In the latter system, we injected the lentiviral vector carrying the gene encoding tetanus toxin light chain gene downstream of the tetracycline-responsive element into the striatum, and then injected AAV vector carrying the tetracycline reverse-transactivator gene into the nigra. We observed that motor deficits were induced by doxycycline administration in the monkeys injected with the vectors. The present data indicate that the application of the NeuRet vector enables us to manipulate a particular neuronal population in primates.

(COI: No)

S52-3

Neuronal activities in the motor thalamus of Parkinsonian rats: Rate vs Pattern

Nakamura, Kouichi C.[1,2]; Sharott, Andrew[2]; Vinciati, Federica[2]; Tanaka, Takuma[3]; Mallet, Nicolas[2]; Magill, Peter J.[2] ([1]*Grad Sch Med, Kyoto Univ, Kyoto, Japan;* [2]*MRC ANU, Oxford Univ, Oxford, UK;* [3]*Interdiscipl Grad Sch Sci Eng, Tokyo Inst Technol, Tokyo, Japan*)

The cerebral cortex, basal ganglia and thalamus together form looping circuits that are critical for movement. Some cardinal movement difficulties of Parkinson's disease (PD) are associated with the abnormal neuronal oscillations, particularly at beta frequencies (13-30 Hz), in the cortex and basal ganglia. Neurons of the motor thalamus are key mediators of basal ganglia influences on cortical motor processing. The motor thalamus is parcellated into two major zones; the 'basal ganglia-recipient zone' (BZ) and the 'cerebellar-recipient zone' (CZ). To address the key issue of how their activities might be disturbed in PD, we recorded the spontaneous firing of identified BZ and CZ neurons under anesthesia in dopamine-intact rats as well as PD model rats prepared by unilateral 6-hydroxydopamine (6-OHDA) lesion of midbrain dopamine neurons. In the PD rats, we found no evidence of pathologically reduced firing rates in either zone of motor thalamus. However, the tight coupling of firing of BZ neurons to cortical slow oscillations was phase-shifted by ~100 degrees. During cortical activation, many BZ neurons, but not CZ neurons, exhibited abnormal beta oscillations in the PD rats. Moreover, blockade of BZ thalamocortical activity by local GABA infusion swiftly abolished the ongoing beta oscillations in the cortex. We conclude that the thalamocortical substrates of movement difficulties in PD are more closely related to abnormal firing patterns than altered firing rates.

(COI: No)

S52-4

Functional thalamic inputs to the primary motor cortex during voluntary movements

Tanaka, Yasuyo H[1,4]; Tanaka, Yasuhiro R[1,5]; Wake, Hiroaki[2]; Hira, Riichiro[1,5]; Kondo, Masashi[1,4,5]; Masamizu, Yoshito[1,5]; Kawaguchi, Yasuo[3,5]; Matsuzaki, Masanori[1,5] ([1]*Div Brain Circuits, National Institute for Basic Biology, Okazaki, Japan;* [2]*Div Homeostatic Development Dept Developmental Physiol, National Institute for Physiological Sciences, Okazaki, Japan;* [3]*Div Cerebral Circuitry, National Institute for Physiological Sciences, Okazaki, Japan;* [4]*Physical and Health Edu, Gradu School of Edu, The Univ of Tokyo, Tokyo Japan;* [5]*JST, CREST, Saitama, Japan*)

Thalamocortical (TC) pathways drive information processing in the cerebral cortex. In the primary motor cortex (M1), TC inputs are known to carry information of the basal ganglia and the cerebellum to finally give an impact on the corticospinal neurons, which send motor signals into the spinal cord. However, it remains unknown how TC pathways drive M1 to execute a voluntary movement. To address this issue, using two-photon microscopy and an adeno-associated virus that encodes GCaMP6 we visualized the neuronal activity of TC axons projecting to M1 during a self-initiated lever-pull task. We found that the TC axons showed sequential activities. Next, to test functional contribution of TC axonal inputs to M1, we perturbed the activity of channelrhodopsin-2-expressing TC axons during the lever-pull task by blue-light illumination on the cortical surface of M1. We found that the task performance of mice became worse during the photostimulation period. In addition, by a photostimulation mapping *in vitro* we found that TC axons directly innervated corticospinal neurons. These results suggest that TC axons send a critical signal to M1 to execute lever pulling.

(COI: No)

S52-5

Functional activity in motor cortex and striatum for voluntary movements

Isomura, Yoshikazu (*Brain Sci Inst, Tamagawa Univ, Tokyo, Japan*)

The primary motor cortex and its major subcortical target, dorsolateral striatum, play a crucial role in controlling voluntary movements as an entrance of the skeletomotor loop. The layer 5 pyramidal cells in the primary motor cortex send glutamatergic axons to the medium spiny neurons in the striatum, the only types of projection neurons, which send GABAergic outputs to other parts of the basal ganglia. The striatal projection neurons participate in either the direct pathway (expressing dopamine D1 receptors) or the indirect pathway (D2 receptors). These D1- and D2-expressing neurons should be excited and inhibited, respectively, by dopamine release from the substantia nigra neurons, encoding a reward prediction error. Many researchers believe that the activation of direct pathway enhances voluntary movements (like as an accelerator in a car), while the activation of the indirect pathway depresses them (as a brake). However, it remains unclear how differentially individual striatal neurons for the two pathways represent motor information, and whether the motor information is affected by reward expectation. Recently, we examined spike activity of single striatal neurons, which were identified juxtacellularly with in situ hybridization, and also of motor cortex neurons by multi-neuronal recordings, in the rats performing voluntary forelimb movements in a reward-expectable condition. Our observations suggest that striatal neurons for the two pathways may work coordinately to integrate motor and reward information, while motor cortex neurons may specifically process motor information.

(COI: No)

Symposium 53

Synaptic structure and (dys) function: How do synaptologists challenge brain disease?

(March 23, 13:30～15:00, Room C)

S53-1

Synapse protection as a novel therapeutic strategy for psychiatric diseases

Hayashi-Takagi, Akiko (*Lab of Structural Physiol, Grad Sch Med, Umin Univ, Tokyo, Japan*)

Drug discovery in psychiatry has been limited to chemical modifications of compounds originally discovered serendipitously. Therefore, more mechanism-oriented strategies of drug discovery for mental disorders are awaited. Schizophrenia (SZ) is a devastating mental disorder with synaptic disconnectivity involved in its pathophysiology. Reduction in the dendritic spine density is a major alteration that has been reproducibly reported in the cerebral cortex of patients with SZ. Disruptedin-Schizophrenia-1 (DISC1), a factor that influences endophenotypes underlying schizophrenia, has a regulatory role in the postsynaptic density in association with the NMDA-type glutamate receptor, and Rac1. Prolonged knockdown of DISC1 leads to synaptic deterioration, reminiscent of the synaptic pathology of SZ. Thus, we tested the effects of novel inhibitors to p21-activated kinases (PAKs), major targets of Rac1, on synaptic deterioration. PAK inhibitors prevented progressive synaptic deterioration in adolescence as shown by in vivo two-photon imaging and ameliorated a behavioural deficit in prepulse inhibition in adulthood in a DISC1 knockdown mouse model. The beneficial effects of synaptic protection by low-molecular weight compound, including PAK inhibitors reported here, may provide us with an opportunity for drug discovery in major mental illnesses with synaptic disturbance. Thus, in the last section of my talk, I will show the recently established high-throughput compound screening by the simultaneous measurement of key disease-related parameters such as glutamatergic synapse, palvalbumin interneuron, and oxidative stress.

(COI: No)

S53-2

CDC42EP4/septin-based perisynaptic glial scaffold that facilitates glutamate clearance

Ageta-Ishihara, Natsumi[1]; Yamazaki, Maya[2]; Konno, Kohtarou[3]; Nakayama, Hisako[4]; Abe, Manabu[2]; Hashimoto, Kenji[5]; Nishioka, Tomoki[6]; Kaibuchi, Kozo[6]; Miyakawa, Tsuyoshi[7,8]; Hashimoto, Kouichi[4]; Watanabe, Masahiko[3]; Sakimura, Kenji[2]; Kinoshita, Makoto[1] (*[1]Dept Mol Biol, Grad Sch Sci, Nagoya Univ, Nagoya, Japan; [2]Dept Cell Neurobiol, Brain Res Inst, Niigata Univ, Niigata, Japan; [3]Dept Anat, Grad Sch Med, Hokkaido Univ, Sapporo, Japan; [4]Dept Neurophysiol, Grad Sch Biomedical Sci, Hiroshima Univ, Hiroshima, Japan; [5]Cent Forensic Mental Hlth, Chiba Univ, Chiba, Japan ; [6]Dept Cell Pharm, Grad Sch Med, Nagoya Univ, Nagoya, Japan; [7]Cent Gene Anal of Behavior, NIPS, Okazaki, Japan; [8]Div System Med Sci, Fujita Health Univ, Toyoake, Japan*)

CDC42EP1-5/BORG1-5, a family of small GTPase effector proteins, interact with CDC42 or heterooligomers of septin GTPases in a mutually exclusive manner, but the physiological significance is unknown. We find that CDC42EP4 is expressed in the mouse cerebellar cortex exclusively in Bergmann glia, where CDC42EP4 localizes to specific membrane domains enwrapping dendritic spines of Purkinje cells. Proteomic analyses of cerebellar lysate indicate that CDC42EP4 is in complex with septin heterooligomers composed of SEPT2/3/4/5/6/7/8/10/11, and the glutamate transporter GLAST, but not with CDC42. In *Cdc42ep4*$^{-/-}$ mice, GLAST is physically dissociated from the septins, and GLAST signals on perisynaptic glial membranes are delocalized away from parallel fiber-Purkinje cell synapses. Patch-clamp analysis of *Cdc42ep4*$^{-/-}$ cerebellar slices reveals that the inward current to Purkinje cells following parallel fiber stimulation is mildly protracted after blocking AMPA receptor desensitization, and surges severely upon additional inhibition of glutamate transporters. These data indicate that glutamate clearance capacity in *Cdc42ep4*$^{-/-}$ Bergmann glia is compromised, which is partially compensated by two adaptive mechanisms. The glutamate intolerance manifests *in vivo* as motor learning defects in the balance beam test. Together, the unique phenotype indicates that CDC42EP4-dependent interaction with septins facilitates perisynaptic concentration and efficiency of GLAST to achieve sufficient buffering and clearance of glutamate, and motor coordination.

(COI: No)

S53-3

Epigenetic regulation of homeostatic synaptic plasticity under epileptic neuronal activity

Futai, Kensuke[1]; Mao, Wenjie[1]; Watanabe, Takuya[1]; Shen, Li[2]; Hock, Hanno[3]; Akbarian, Schahram[2] (*[1]BNRI, Dept Psychiatry, Univ Mass Med Sch, MA, U.S.A.; [2]Dept Psychiatry, Mount Sinai Sch Med, NY, U.S.A.; [3]Dept Medicine, Massachusetts General Hospital, MA, U.S.A.*)

Adjusting to runaway network excitation is critical to prevent neuronal cell death in epilepsy and requires rapid induction of a series of genes by synaptic activity. Understanding the regulatory mechanism underlying network excitation-dependent gene expression has broad implications, as chronic shifts in the excitatory and inhibitory balance (E/I balance) are found in many neurological disorders, including epilepsy, Autism Spectrum Disorders, Schizophrenia and Alzheimer's disease. The mechanisms of homeostatic synaptic plasticity, including downscaling of the strength of excitatory synaptic transmission in neurons, should play a critical role in preventing runaway excitation by maintaining the E/I balance. The expression of this plasticity requires neuronal activity-dependent gene transcription and protein synthesis. Gene expression, in turn, is affected by complex chromatin remodeling and histone modification machinery. Intricate post-transcriptional modifications of histones change the conformation of chromatin, and, thus, determine the active or inactive states of gene expression. These modifications are achieved by chromatin remodelers. However, the role of chromatin remodeling in homeostatic synaptic plasticity is largely unclear. In this session, I will discuss our recent results addressing the role of chromatin remodeler on homeostatic synaptic plasticity.

(COI: No)

S53-4

Molecular mechanisms for altered spine dynamics among ASD model mice

Fukumoto, Keita[1,2]; Tamada, Kota[1]; Tanaka, Shinji[3]; Okabe, Shigeo[3]; Takumi, Toru[1,2,4] (*[1]RIKEN Brain Science Institute., Saitama, Japan; [2]Grad.Sch. Biomed Sci. Hiroshima Univ., Hiroshima, Japan; [3]Grad.Sch.Med. Tokyo Univ., Tokyo, Japan; [4]CREST, JST*)

Autism spectrum disorder (ASD) is a neuropsychiatric disorder appeared in childhood when synaptic dynamics is highly active. Many CNVs (copy number variation) / SNVs (single nucleotide variation) of synaptic molecules are found in patients with ASD. In addition, model mice for ASD show a wide variety of abnormal synaptic changes in light of density, length and maturity, etc., but there have been no common abnormality among them.We generated a CNV mouse model for ASD (patDp/+) by chromosome engineering technique. patDp/+ mice in which the duplication of mouse chromosome 7 (corresponding to human 15q11-q13 region) is derived from paternal allele show abnormal social behavior. We have recently discovered the increased turnover rate of spines in the cerebral cortex of patDp/+ mice as a common endophenotype among mouse models of ASD. To identify the responsible gene(s) for the spine phenotypes, we systematically searched them using in vivo imaging by introducing each gene in the 15q11-13 region. We will present our progress on this screening and the implication of the candidate gene on spine functions.

(COI: No)

S53-5

Circadian genes, rhythms and biology of mood disorders

McClung, Colleen (*Univ Pittsburgh*)

Disruptions in sleep and circadian rhythms are one of the central features of several psychiatric diseases including bipolar disorder, major depression and addictive disorders. In fact, changes to the sleep wake cycle are one of the core symptoms used for diagnosis of these diseases. Moreover, several human genetic studies have identified polymorphisms in the genes that control circadian rhythms that associate with these disorders. However, the mechanisms by which circadian genes control mood, reward, motivation and anxiety remain unclear. Our lab has used mouse models to try to uncover some of the molecular and cellular mechanism by which circadian gene disruption leads to changes in reward and mood. Importantly, these behaviors can be normalized with both lithium and valproic acid treatment, adding face validity to this model. More recently, we found that these mice cycle throughout a 24 hour period with manic-like behavior becoming apparent during the light phase and the return to a euthymic-like state during the dark phase. To the best of our knowledge, this is the only mouse model which spontaneously switches mood-related states. This model allows us to probe the neurobiology and molecular mechanisms that underlie the switch to a manic state in bipolar disorder. These mechanisms, along with the mechanisms by which mood stabilizing drugs produce a therapeutic response in these mice will be discussed.

(COI: Properly Declared)

Symposium 54

Recent findings in development, function and disease of GABAergic neurons

(March 23, 13:30～15:00, Room D)

S54-1

The multi-faced GABA: role of GABA signaling in basic developmental processes in and outside the nervous system

Szábo, Gábor (*Institute of Experimental Medicine, HAS, Budapest, Hungary*)

GABA is present in the whole living kingdom from bacteria through yeast and plants to vertebrates with diverse, but also shared basic signaling functions.
In mammals, GABA is mostly recognized as the principal inhibitory neurotransmitter, however it is also present in a wide variety of peripheral tissues. During development both in the nervous system and other organs, GABA regulates basic processes including proliferation, differentiation and migration. The diverse action of GABA is underlined by the molecular complexity of its signaling components including the synthesizing enzymes (adult and embryonic GADs), receptors and transporters.
Here we describe the presence and the role of GABA signaling in undifferentiated ES cells that corresponds to the blastocyst developmental stage, where it modulates proliferation and differentiation through GABAA and GABAB receptors in an opposite way by regulating intracellular calcium levels. For the first time, we detected all GAD forms, a variety of GABA receptor subunits and both membrane and vesicular GABA transporters in the developing eye lens, where they are expressed in the fiber cells in a spatially and temporally regulated fashion. Using mouse models with genetically altered GAD levels and primary lens cultures, we determined that different GAD forms have distinct functions in fiber cell proliferation, differentiation and elongation. In these processes GABA also acts through intracellular calcium rise.
The developmental role of GABA signaling in the formation and migration of the GnRH neuronal system will also be discussed.

(COI: No)

S54-2

Subclass-specific expression of perineuronal nets around parvalbumin-expressing GABAergic neurons in the mouse hippocampus

Yamada, Jun (*Grad.Sch.Med.Sci., Kyushu Univ., Fukuoka, Japan*)

The perineuronal nets (PNNs) surround the soma of a subset of neurons, and is considered to play a critical role in regulation of neural plasticity. Seminal works using immuno/lectin histochemistry reported that PNNs were associated with parvalbumin-expressing (PV+) GABAergic neurons. Although later reports have shown that PV+ neurons consist of at least five subclasses (basket cells, axo-axonic cells, bistratified cells, oriens-lacunosum-moleculare (O-LM) cells and hippocampo-septal projection (H-S) cells), the relationship between PNNs and classification of PV+ neurons remains unclear. We clarify whether PNNs are associated with specific subclasses of PV+ neurons in the mouse hippocampus. To characterize PNNs, we used Wisteria floribunda agglutinin (WFA) lectin and the antibodies against aggrecan (core protein), hyaluronan and proteoglycan link protein 1 (HAPLN1; link protein) and Cat-315 epitope (chondroitin sulfate proteoglycan). The PNNs labeled by WFA were associated with PV+ basket cells. The PNNs defined by Aggrecan were found in basket cells, O-LM cells and H-S cells, while HAPLN1-positive PNNs and Cat-315-positive PNNs were associated with basket cells and H-S cells. These data provide compelling evidence that PNNs may critically regulate hippocampal neuronal activity via subclass specific expression in PV+ neurons.

(COI: No)

S54-3

Prenatal stress-induced selective deterioration of neurogenesis of parvalbumin-positive GABAergic neurons

Fukuda, Atsuo (*Dept Neurophysiol., Hamamatsu Univ Sch Med., Hamamatsu, Japan*)

Exposure to prenatal stress and mutations in GAD1, which encodes the γ-aminobutyric acid (GABA) synthesizing enzyme glutamate decarboxylase (GAD) 67, are both risk factors for psychiatric disorders. In addition, decrement of parvalbumin (PV)-positive GABAergic interneurons in the medial prefrontal cortex (mPFC) and hippocampus (HIP) has often been observed in schizophrenia patients. However, the relationship between these risk factors remains unclear. So we examined GAD67-green fluorescent protein (GFP) knock-in mice (i.e., mice in which the Gad1 gene is heterozygously deleted; GAD67+/GFP) that underwent prenatal stress from embryonic day 15.0 to 17.5 to address the interaction between Gad1 disruption and stress. Administration of 5-bromo-2-deoxyuridine revealed that neurogenesis of GFP-positive GABAergic neurons, but not cortical plate cells, was significantly diminished in GAD67+/GFP but not in wild type (GAD67+/+) fetal brains during maternal stress. Differential expression of glucocorticoid receptors by different progenitor cell types may underlie this differential outcome. Postnatally, the density of PV-positive, but not PV-negative, GABAergic neurons was significantly decreased in the mPFC, HIP and somatosensory cortex of GAD67+/GFP mice. By contrast, these findings were not observed in GAD67+/+ offspring. These results suggest that prenatal stress, in addition to heterozygous deletion of Gad1, could specifically disturb the proliferation of neurons destined to be PV-positive GABAergic interneurons (Uchida et al., Transl. Psychiatry 2014).

(COI: No)

S54-4

Glutamate decarboxylase deficiency displays schizophrenia-like phenotypes: a study using knockout mice

Yanagawa, Yuchio (*Department of Genetic and Behavioral Neuroscience, Gunma University Graduate School of Medicine, Maebashi, Japan*)

GABA is a major inhibitory neurotransmitter in the adult mammalian CNS. GABA is synthesized by two forms of glutamate decarboxylases (GAD65 and GAD67). Impairments in GABAergic neurotransmission are thought to be associated with the pathogenesis of psychiatric disorders such as anxiety disorder, epilepsy and schizophrenia. For example, postmortem brain studies of schizophrenia patients have revealed GAD67 reduction in parvalbumin (PV) neurons of the cerebral cortex and hippocampus. To develop a mouse model of the psychiatric disorders and to further elucidate the involvement of GABAergic dysfunction in the disorders at molecular level, we generated conventional GAD65 and GAD67 knockout (KO) mice. Whereas the GAD65 KO mice displayed increased level of anxiety and spontaneous seizures, the GAD67 KO mice died around birth probably due to cleft palate and respiratory failure. Considering the perinatal death of the conventional KO mice, we generated conditional GAD67 KO mice, in which GAD67 was deficient primarily in PV neurons, and named these KO mice PV-GAD67 mice. The PV-GAD67 heterozygous mice displayed schizophrenia-like phenotypes such as an increase in MK801-induced locomotor activity and a deficit in prepulse inhibition, indicating that GAD67 reduction is associated with the pathophysiology of schizophrenia. The approaches using our KO mice will be discussed.

(COI: No)

Symposium 55

New roles for biological clocks in homeostasis

(March 23, 13:30～15:00, Room F)

S55-1

Homeostatic regulation of the circadian clock system evaluated by in vivo whole body imaging

Tahara, Yu (*Dept. of Physiol. and Pharm., Sch. of Adv. Sci. and Engi., Waseda Univ.*)

The circadian clock systems in mammals, endogenous pacemakers located in the brain and periphery, organizes various biological activities, and are synchronized by the environmental factors, such as light-dark cycles, food, exercise, and drugs. Recently, we have developed new methodology to measure gene expression rhythms of peripheral clock by in vivo imaging system using PER2::LUCIFERASE knock-in mice. This method enables us to track the changes of circadian network among tissues in individual mouse for long-term. Using this, we focused on the stress response of the circadian clocks, and found that physical/psychological stress was a potent synchronizer of peripheral clocks. Stress stimuli induced time-of-day dependent phase-advance or -delay of clock gene expression rhythms in peripheral tissues, as well as in the hippocampus and cortex, but not in the suprachiasmatic nucleus, a central clock. Moreover, several days of stress exposure at beginning of the light period caused loss of oscillation and severe internal desynchronization of peripheral clocks among tissues. This response was produced through glucocorticoid and sympathetic nervous activations with acute/direct change of clock gene expressions in peripheral tissues. Additionally, this response could be habituated and disappeared by repeated stress exposures. Thus, our results newly demonstrated that acute stress caused severe change of peripheral clocks, and also stress was a strong entrainable factor for the peripheral clocks.

(COI: No)

S55-2

Seasonal rhythms in affective states and communication between brain and peripheral metabolism

Yasuo, Shinobu (*Fac. Agr. Kyushu Univ., Fukuoka, Japan*)

Mammals have adapted their physiological functions and behaviors to seasonal changes in environment by using photoperiod as a primary cue. Humans also exhibit seasonality in their mood, sleep, sociality, appetite, and energy balance, which may be a vestige of evolutionally adaptation mechanisms. Extreme seasonality causes seasonal affective disorder (SAD) marked by depression during specific seasons, generally winter. Symptoms of SAD also include hypersomnia, hyperphagia, weight gain, and carbohydrate craving, suggesting that metabolic changes associating with brain functions are underlying SAD. This hypothesis has not been addressed experimentally using laboratory rodents, because mice and rats are non-seasonal breeders and have been considered as inappropriate models of SAD. In recent years, we clarified that exposure of C57BL/6J mice to winter-like photoperiod induces high immobility in forced swim test, a depression-like behavior, and low preference for saccharine, a depression-related anhedonia, accompanying low levels of brain serotonin. Interestingly, these mice under winter-like photoperiod also exhibited fat accumulation, alteration of plasma composition of free amino acid levels, low glucose tolerance, and alteration of muscle physiology. Further, photoperiod regulated corticosterone release that is deeply associated with peripheral metabolism, and adrenalectomy abolished the photoperiod-dependent changes in depression-like behavior. In the presentation, involvement of communication between peripheral metabolism and brain functions in seasonal regulation of mood will be discussed.

(COI: No)

S55-3

Hypothalamic regulation of energy metabolism by feeding rhythm

Shiuchi, Tetsuya[1,2] ([1]*Dept. Integrative Physiology, the Univ. of Tokushima Graduate School, Tokushima, Japan;* [2]*PRESTO, JST, Saitama, Japan*)

Biological clock is modulated by not only light stimulation also feeding stimulation. It has been recognized that disturbance of feeding rhythm has been understood as a risk factor for development of insulin resistance because of mess up the timing of expression phase of clock genes especially in liver. In contrast, hypothalamic control of peripheral energy metabolism is an important regulatory system in whole body homeostasis, although effect of feeding rhythm on it has not elucidated. We separated C57BL/6J mice for 3 feeding scheduled groups; given lab chows freely during dark phase (ZT12-24, Control group), first 4-hour in dark phase (ZT12-16; Morning group), and last 4-hour in dark phase (ZT20-24, Evening group). Mice in Evening group showed impaired whole body insulin sensitivity despite the smaller food intake than that of Control group, while mice in Morning group showed normal insulin sensitivity. We observed that higher lipid accumulation, increased gene expression of fatty acid synthesis, decreased fatty acid oxidation and impaired insulin signals in skeletal muscle in Evening group compared to other group. These effects were not observed in liver. On the other hand, mRNA expression of agouti-related protein (AgRP) was increased in hypothalamus in Evening group. Inhibition of central AgRP expression by antisense oligo improved insulin resistance in whole body and skeletal muscle of Evening group. These results suggest that feeding rhythm like as ingestion only in the evening impairs insulin sensitivity in whole body and skeletal muscle mediated by hypothalamic AgRP.

(COI: No)

S55-4

Exploring the mechanism of homeostasis: A study on a common kinase regulating metabolism and circadian clock

Hayasaka, Naoto[1,2] ([1]*Yamaguchi Univ. Grad. Sch. Med., Ube, Japan;* [2]*PRESTO, JST, Kawaguchi, Japan*)

Circadian clocks are known to play a role in the regulation of homeostasis, and their impairment may lead to disorders such as metabolic, immune and neuropsychiatric diseases. We found salt-inducible kinase 3 (SIK3) to be an essential component of both metabolism and circadian clock. *Sik3*-deficient (*Sik3* KO) mice demonstrate severe defects in glucose, lipid, bile acid and vitamin A metabolisms, and over 90 % of homozygotes die postnatally. In addition, *Sik3* KO mice exhibit several abnormalities in circadian rhythms, e.g., significantly longer and unstable periods in behavioral rhythms, imperfect entrainment to light-dark cycles, and splitting of light-entrained and non-entrained (free-running) rhythms in behavior under light re-entrained condition. These data, along with a previous report indicating that SIK3 is evolutionally very well-conserved from nematodes to mammals, strongly suggest an indispensable role for SIK3 in homeostasis and survival. To address the question of whether SIK3 mediates an interaction between metabolism and circadian clock to maintain homeostasis, we generated conditional *Sik3* KO mice and found a link between circadian clock and metabolic homeostasis.

(COI: No)

S55-5

Insulin mediates feeding-induced circadian entrainment in liver and white adipose tissue

Sato, Miho[1]; Hayasaka, Naoto[2,3]; Akashi, Makoto[1] ([1]*Res Inst for Chronobiology, Yamaguchi Univ, Yamaguchi, Japan;* [2]*Yamaguchi Univ. Grad. Sch. Med., Ube, Japan;* [3]*PRESTO, JST, Kawaguchi, Japan*)

The circadian clock is entrained to environmental cycles. Although the light input pathway has been well characterized, the mechanism of feeding-induced phase adjustment remains unclear. Here, we focused on insulin as one of the endogenous molecules and report that insulin may be involved in feeding-induced entrainment in vivo. To help elucidate the in vivo roles of insulin, we used S961, which is a highly specific competitive peptide inhibitor of insulin. Subcutaneous injection of S961 inhibited feeding-induced immediate early expression of Per2 transcripts in liver. Also, attenuation in the circadian entrainment of the liver clock was statistically significant in S961-treated mice. In ex vivo culture experiments, insulin induced phase shift in liver and white adipose tissue. Furthermore, the direction of the phase shift was phase specific, phase delay during PER2-decreasing phase and phase advance during the increasing phase. These results suggest that insulin maybe an immediate early factor in feeding-mediated tissue-specific entrainment. In this talk, I would like to discuss the significance of the phase regulatory role of glucose-homeostasis-maintaining hormone.

(COI: No)

Symposium 56

Cutting-edge *in vivo* nano-imaging technologies

(March 23, 13:30～15:00, Room G)

S56-1

Toward high-speed high-resolution 3D imaging

Mimori, Yuko Kiyosue[1]; Shimozawa, Togo[1,2] (*[1]Riken CDB; [2]Waseda Univ*)

In this talk, we introduce two advances in spinning disc confocal- and light sheet-based microscopic technologies.
1. Spinning disk confocal microscopy for thick specimens
Yokogawa Confocal Scanner Unit (CSU), containing a set of ~20,000 microlenses and confocal pinholes, offers high-speed multipoint scanning. But conventional model was not optimized for thick specimens. We improved this method using two-photon excitation and modified CSU with a larger pinhole interval. Our strategies dramatically improve higher-resolution intravital imaging of tissues and embryos. (Shimozawa, T. et al., PNAS, 110: 3399-3404, 2013)
2. Lattice light-sheet microscopy for isotropic 3D imaging
Recently, the use of scanned Bessel beams showed to create very thin light sheets (< 300 nm). With the latest model employing lattice light-sheet illumination to achieve faster scanning, we succeeded to track growth of tens of microtubules within the mitotic spindle and all of the chromosomes in 3D, demonstrating its suitability for 3D isotropic high-resolution live imaging. (Chen et al., Science, 2014, in press)

(COI: No)

S56-2

Fast and wide field-of-view live imaging of whole organism by light-sheet microscopy

Nonaka, Shigenori (*NIBB, Okazaki, Japan*)

Light-sheet microscopy is an emerging technology that uses thin sheet-shaped excitation light to illuminate the focal plane of a detection objective. This method is characterized by low bleaching and phototoxicity, deep penetration depth, and high-speed image acquisition. These features are extremely suitable for live imaging of whole organisms of submillimeter scale.We have applied this microscopy for long term and high time-resolution live imaging of gastrulating mouse embryos and freely moving *Amoeba proteus*. Recently we have developed a new two-photon light-sheet microscope that enables better penetration depth and wider field of view, by use of high pulse-energy femtosecond fiber laser.

(COI: No)

S56-3

Relationship between synapse nanostructure and its stability in the mouse neocortex

Tanaka, Shinji[1,2]; Iida, Tadatsune[1,2]; Iwasaki, Hirohide[1,2]; Okabe, Shigeo[1,2] (*[1]Grad. Sch.Med.UnivTokyo., Tokyo, Japan; [2]CREST, JST, Saitama, Japan*)

Two-photon microscopy has advantages over conventional microscopy in deeper tissue penetration of infrared light and less photo-damage. Application of this technique to living animals enables us to observe the dynamics of subcellular structures and molecules in vivo. However, due to the relatively large point-spread function of the infrared light focused by a water-immersion objective lens, the optical resolution of two-photon imaging is not optimal. In order to clarify the relationship between synapse nanostructure and its stability in vivo, new technologies of correlative microscopy should be developed. In vivo two-photon imaging of dendritic spines and postsynaptic molecules revealed that stabilization of spine synapses takes place during the early postnatal development. Most of spine synapses contacting the intracortical axons are highly dynamics until the third postnatal weeks. In contrast, spine synapses receiving thalamic inputs were larger and highly stable. To further characterize morphological details of the dynamic and stable spines, we performed retrospective analyses of synaptic structure imaged in vivo by using CLARITY, which enables rapid access of exogenous antibodies, large volume imaging, and use of an objective lens with a high numerical aperture. This technical development should be useful in analyses of morphological characteristics of both spine synapses and surrounding glial processes with a submicron resolution, which will lead to identification of parameters important for synapse stability.

(COI: No)

S56-4

In vivo visualization of sarcomere dynamics in the beating mouse heart

Kobirumaki-shimozawa, Fuyu; Fukuda, Norio (*Dept Cell Physiol, The Jikei Univ Sch of Med, Tokyo, Japan*)

A fundamental principle in cardiovascular science is that a change in myocardial length dramatically changes the heart's pump functions on a beat-to-beat basis (*Frank-Starling Law of the Heart*). Despite the importance of accurate measurement of sarcomere length in cardiomyocytes, such measurement has not been achieved in the beating heart *in vivo* under the physiological condition, due to technical difficulties. In the present study, therefore, we developed a high-speed high-resolution imaging system for myocardial sarcomeres in living mice. The spatial resolution in the measurement of single sarcomere lengths was 20 nm at 100 frames per second. This system enabled three-dimensional analyses of sarcomere dynamics during the cardiac cycle, simultaneously with electrocardiogram and left ventricular pressure measurements. Since the discovery of the *Law* by Frank and Starling at the turn of the 20th century, we for the first time directly quantified sarcomere length values in left ventricular myocytes *in vivo* under the physiological condition. Likewise, the left ventricular developed pressure was linearly correlated with the sarcomere length change between diastole and systole on the order of 100 nm, providing direct evidence for the tight coupling between sarcomere length and the heart's pump function *in vivo*. The present experimental system has a broad range of application possibilities for unveiling sarcomere dynamics in cardiomyocytes *in vivo* in health and disease.

(COI: No)

S56-5

High accuracy nano-imaging of cancer and peripheral artery disease with X-ray and fluorescence

Gonda, Kohsuke (*Dept Med Phys, Grad Sch Med, Tohoku Univ, Sendai, Japan*)

We have been developing the technologies for X-ray computed tomography (CT) or fluorescence imaging to clarify the mechanism and develop the diagnostic methods for cancer and arterial sclerosis. The gold nanoparticles coated with PEG chains were prepared as contrast agents for X-ray CT imaging. As the resolution of X-ray CT imaging is around several tens of micrometers, X-ray CT can visualize at levels ranging from small tissues to whole body by high penetrative power of X-rays. Quantum dots (QDs) is one of recently-developed fluorescence nanoparticles. To perform various biomedical fluorescence imaging, we are using modified QDs and own in vivo imaging system with spatial accuracy of 9 nm. Fluorescence imaging has the resolution with hundreds of nanometer and high quantitative sensitivity because the fluorescence signal intensity is proportional to the intensity of the photon excitation energy. However, as the fluorescence imaging was affected by optical scatter and absorption in cells or tissues, tissue permeability of fluorescence is not well. Therefore, fluorescence imaging is suitable to visualize at levels ranging from molecular to small or thin tissues. The technology integration of both advantages for X-ray CT and fluorescence imaging is thought to greatly contribute to development of medical imaging with high accuracy and highly-quantitative sensitivity at levels ranging from molecular to whole body. Here we introduce high accuracy imaging of cancer and peripheral artery disease with X-ray CT or fluorescence and discuss the technology integration of both imaging.

(COI: No)

Symposium 57

Neurogenesis from embryo to adult

(March 23, 13:30～15:00, Room H)

S57-1

Oscillatory Expression of bHLH Transcriptional Factors in Neural Stem Cells

Imayoshi, Itaru[1,2]; Kageyama, Ryoichiro[2] (*[1]The Hakubi Center, Kyoto, Univ., Japan; [2]The Institute for Virus Research, Kyoto, Univ., Japan*)

During neural development and in the adult brain, neural stem cells give rise to appropriate numbers of neurons, astrocytes and oligodendrocytes in the specific timing and places. Many important intrinsic and extrinsic factors regulating the fate determination of neural stem cells have been identified, however, how do these key factors or molecules regulate diverse responses of neural stem cells is not still unclear. The basic-helix-loop-helix factors Ascl1/Mash1, Hes1, and Olig2 regulate the fate choice of neurons, astrocytes, and oligodendrocytes, respectively; however, these factors are coexpressed by neural stem cells. How such fate determination factors behave in progenitors and differentiating cells remains elusive. In this study, we found, by time-lapse imaging, that these factors are expressed in an oscillatory manner by neural stem cells, and that one of them becomes dominant during fate choice. Furthermore, FACS sorting and differentiation assay of NPCs having various amount of bHLH factors revealed that neural stem cells had the differentiation biases at that time, and that differentiation biases were dynamically changing by oscillatory expression of bHLH factors. These results indicate that neural stem cells are dynamically changing their state by oscillatory expression of fate determinate factors. We propose new neural stem cell regulatory mechanism that oscillatory expression of bHLH transcriptional factors ensures self-renewable and multi-potent ability of neural stem cells.

(COI: No)

S57-2

From embryonic to adult neurogenesis in the hippocampus

Seki, Tatsunori (*Hist. Neuroanat. Tokyo Med. Univ., Tokyo, Japan*)

In most of brain regions, neurogenic stem cells occur only during embryonic and early postnatal stages, and disappear at adult stage. However, the hippocampus possesses stem cells to continue to produce dentate granule cells from embryonic to adult stages. The adult stem cells are astrocyte-like cells expressing glial fibrilar acidic protein (GFAP) and brain lipid-biding protein (BLBP), and thus are distinct from embryonic stem cells such as those of neocortical pyramidal cells that express BLBP, but not GFAP. Recently, we have found that embryonic dentate stem/progenitor cells are different from both the neocortical and the adult dentate stem/progenitor cells. The embryonic dentate stem/progenitors express GFAP, but not BLBP. The analysis using Gfap-GFP mice and time-lapse imaging revealed that the Gfap-GFP+ distinct cell population first appears in the VZ of the medial pallium at the dorsal edge of the fimbria. During the embryonic period, they form a migratory stream from the VZ to the developing dentate gyrus, and establish the proliferative zones in which Gfap-GFP+ progenitors produce granule cells. Before birth, the Gfap-GFP+ progenitors were mostly negative for BLBP. However, after birth the Gfap-GFP+ progenitors begin to express BLBP, and the number of Gfap-GFP+/BLBP+ progenitors rapidly increase. By P14 a half of progenitors became double positive for Gfap-GFP and BLBP. They were mostly seen in the subgranular zone, and had a radial process, a morphological trait of adult type progenitors. These results indicate that the property of the dentate progenitors cells is converted immediately after birth, and become adult type progenitors.

(COI: No)

S57-3

Stress that induces adult neurogenesis in the mouse neocortex

Tamamaki, Nobuaki (*Dept of MNS, Kumamoto Univ., Kumamoto, Japan*)

It is well known that a significant number of neurons are continuously produced in the two sites of the adult mammalian brain, the hippocampal dentate gyrus and the subventricular zone of the telencephalon. The dentate gyrus produces new granule cells to code new descriptive memory every day. The subventricular zone of the telencephalon produces new GABAergic neurons as a response to the turn-over of the olfactory receptor cells in the nasal epithelium. Therefore, the adult neurogenesis might be the phenomena generally induced by the stress added on the neuron progenitors.As the results of aging, accidents, and other factors, brain suffers from hemorrhage, ischemia, epilepsy, amyloid deposition, virus infection and physical damages. There pathological damage may be stress to the neocortical neuron progenitors hidden somewhere inside the cranium. However, as far as we searched many papers, hemorrhage or ischemia failed to generate new neurons with an axon. Therefore, we started to use the other stress, such as (repetitive electrical stimulation) kindling and virus infection. Kindling induced epileptic seizure at each stimulation and grew the pia-progenitor in the pia mater and arachnid membrane (the leptomeninges). Finally kindling induced a small number of excitatory neurons and GABAergic neurons with an axon. Moreover, elimination of either excitatory neurons or GABAergic neurons by DTA expression induced many BrdU-positive excitatory neurons or BrdU-positive GABAergic neurons, respectively.

(COI: No)

S57-4

Understanding the new neurons in the olfactory bulb within the large olfactory neuronal network

Yamaguchi, Masahiro (*Dept Physiol, Grad Sch Med, Univ Tokyo, Japan*)

New neurons are continually incorporated into the neuronal circuit of the olfactory bulb (OB) even in adulthood. The new neurons differentiate into granule cells (GCs), the major inhibitory neurons in the OB, and provide remarkable plastic potential to the neuronal circuit. In this talk I will explain how new GCs work within the large olfactory neuronal network that involves the OB and the olfactory cortex. Among new GCs, nearly half are incorporated into the neuronal circuit while the other half are eliminated by apoptosis. This cell selection is important for the refinement and fine tuning of the neuronal circuit. New GCs receive bottom-up olfactory sensory inputs from the external world, and also receive top-down inputs from the principal neurons in the olfactory cortex. The bottom-up sensory inputs activate a subset of new GCs, and top-down inputs from the olfactory cortex selectively eliminate new GCs which are not activated by the bottom-up inputs. Thus selection of new GCs is conducted by the integration of bottom-up inputs from the periphery and centrally-generated top-down inputs. Further, I will discuss possible roles of new GCs in the signal transfer between the OB and the olfactory cortex and the proper expression of olfactory behaviors.

(COI: No)

S57-5

Relationship between frontal cortical oligodendrocyte and mood

Hayashi, Yoshitaka[1]; Fuke, Satoshi[1]; Fuchigami, Takahiro[1]; Koyama, Natsu[1]; Tatebayashi, Yoshitaka[2]; Hitoshi, Seiji[1] (*[1]Dept Integrative Physiol, Grad Sch Med, Shiga Univ Med Sci, Shiga, Japan; [2]Dept Affective Disorder, Tokyo Metro Inst Med Sci, Tokyo, Japan*)

In the post mortem brain study, stereological methods were usually used to estimate the number of cells in the brain. However, stereological methods are laborious and intrinsically low throughput, taking typically long periods to complete a large study. We therefore developed a novel quantitative cell-counting method using a flow cytometer. We applied this method to frozen unfixed postmortem human brains of the frontopolar and inferior temporal cortex from patients with mood disorders (major depressive disorders and bipolar disorders) and normal controls. We found significant reductions of the number of oligodendrocyte in the frontopolar cortex of mood disorders. The reduction of oligodendrocyte in frontopolar cortex from patients with mood disorders suggests that the pathogenesis of mood disorders may involve some abnormalities in oligodendrocyte in the frontopolar cortex. To explore further the dynamics of cortical oligodendrocyte, we demonstrated the oligodendrogenesis in the brain from crab-eating monkeys (macaca fascicularis), and estimated the number of oligodendrogenesis in the frontal, temporal, and occipital cortex. We found that the number of oligodendorogenesis was larger in frontal cortex than other region. Furthermore, we try to establish a nonhuman primate model of cytokine-induced depression by using interferon-alpha. In the preliminary study, we observed abnormalities of behavioral and neuropathological changes in the depression model of monkey.

(COI: No)

Symposium 58

Neuronal circuit in the basal ganglia in terms of transmitters and receptors

(March 23, 13:30～15:00, Room I)

S58-1

Area-specific dopamine receptor expression of astrocytes in basal ganglia

Yamada, Katsuya; Nagatomo, Katsuhiro (*Dept Physiol, Hirosaki Univ Grad Sch Med, Aomori, Japan*)

Midbrain substantia nigra pars reticulata (SNr), the major output nucleus of the basal ganglia, receives dopamine via dendrites of dopamine neurons, of which cell bodies locate in the adjacent nucleus substantia nigra pars compacta (SNc). However, cellular elements, especially receptor expression profiles, to which such dendritically released dopamine targets, is yet to be identified completely. Here we show that processes, but not nuclei, of acutely dissociated SNr astrocytes express D1 dopamine receptors by immunocytochemical investigation. No significant D2 receptor expression was detected in SNr astrocytes. In contrast, D1-expressing and D2-expressing astrocytes were found in striatum, although no such expression was detected in astrocytes obtained from cerebral cortex in the same slice. Based on these findings with other information so far obtained, we propose a working hypothesis that astrocytes in basal ganglia express dopamine receptors in their processes in an area-specific manner.

(COI: No)

S58-2

The role of physiologically released dopamine in the striatum

Momiyama, Toshihiko (*Dept Pharmacol, Jikei Univ Sch Med, Tokyo, Japan*)

Dopaminergic neurons in the substantia nigra pars compacta (SNc) send their axons to medium spiny neurons as well as cholinergic interneurons in the striatum, regulating neuronal activities of these striatal neurons. Nigro-striatal dopaminergic pathway plays important roles in motor control through the interaction between dopamine (DA) and acetylcholine (ACh). One of the potential mechanisms underlying the motor control is synaptic transmission in the striatum. However, little information has been available on the DA receptor subtypes contributing to the synaptic transmission.In the present study, Whole-cell patch-clamp analysis was carried out in dopamine (DA) D2 receptor (D2R) knock-out (KO) mice to elucidate the function of this receptor in the regulation of GABAergic synaptic transmission onto striatal cholinergic interneurons. In slice preparation obtained from wild type mice, GABAergic inhibitory postsynaptic currents (IPSCs) showed frequency-dependent suppression, and the suppression significantly reduced in D2R KO mice. Contribution of N-type calcium channel was significantly reduced in the striatal cholinergic interneurons of the D2R KO mice compared with that in the wild type mice, where D2-like receptors and N-type channels are tightly coupled in the GABAergic transmission. These findings provide a concrete evidence for the physiological role of D2R in the regulation of GABAergic synaptic transmission onto striatal cholinergic interneurons, confirming the tight coupling D2R and N-type calcium channels in the regulation of GABA release.

(COI: No)

S58-3

Regional Difference in Network of rat basal ganglia

Fujiyama, Fumino[1,2]; Unzai, Tomo[1]; Mizutani, Kazuko[1]; Oh, Yoonmi[1]; Nakano, Yasutake[1]; Nagai, Wataru[1]; Takahashi, Susumu[1]; Karube, Fuyuki[1]
([1]*Grad.Sch.Brain Sci.Doshisha Univ., Kyoto, Japan;* [2]*CREST, JST*)

Motor coordination and reinforcement learning mechanisms have recently been proposed to work in the neural circuit of the basal ganglia. However, the mechanism has been difficult to confirm anatomically, partially because of the complex organization of the striatum. In particular, because the striosome/matrix compartments are highly irregular and cannot be visualized without processing such as immunostaining, identification of the exact input and output pathways is difficult to be analyzed. We recently elucidated the input/output organization of each compartment using a single neuron reconstruction with viral vectors expressing membrane-targeted fluorescent proteins and other tracing studies. With respect to the excitatory striatopetal afferents, the striatal compartments receive different lines of information from the not only the cortical areas but also the thalamic nuclei. As concerned with the intra- basal ganglia network, not only the striatum but also the globus pallidus showed the characteristic projection patterns toward the other nuclei in relation to the chemical subregions of basal ganglia. These findings revealed that the topographic organization of the striatum could be well correlated with both the cortico-basal ganglia-thalamic loops and the intra- basal ganglia network. It suggests that the specific input/output organizations make possible the precise tuning for the motor coordination and reinforcement learning.

(COI: No)

S58-4

Control of behavioral flexibility by striatal cholinergic interneurons

Kobayashi, Kazuto (*Dept Mol Genet, Fukushima Med Univ, Fukushima, Japan*)

Flexible switching of behaviors in response to changes in environments is essential for the survival of animals. This behavioral flexibility is mediated through the neural circuitry linking the prefrontal cortex and basal ganglia. In the present study, the role of striatal cholinergic interneurons in behavioural flexibility is addressed by eliminating selectively these neurons in transgenic rats by immunotoxin-mediated cell targeting. Elimination of cholinergic interneurons from the dorsomedial striatum (DMS), but not from the dorsolateral striatum, results in enhanced reversal and extinction learning, sparing the acquisition of place discrimination. In addition, gene-specific silencing of M4 muscarinic receptor by lentiviral expression of short-hairpin RNA also enhances the place reversal learning, whereas gene silencing of M1 muscarinic receptor does not affect the performance of reversal learning. These data indicate that DMS cholinergic interneurons play a key role in the inhibition of behavioural flexibility, mainly through the M4 muscarinic receptor.

(COI: No)

S58-5

Role of dopamine D1 receptors in the hippocampal dentate gyrus in the action of antidepressants

Nishi, Akinori (*Dept of Pharmacol, Kurume Univ Sch of Med, Kurume, Japan*)

Antidepressant drugs are widely used for the treatment of depression. Mechanisms of antidepressant action are not fully understood. Recently, a selective serotonin reuptake inhibitor, fluoxetine, is shown to induce functional changes in mature granule cells in the hippocampal dentate gyrus (PNAS 107:8434-8439, 2010), in addition to the facilitation of adult neurogenesis. The profiles of granule cell functions after chronic fluoxetine treatment resemble to those of immature dentate gyrus in alpha-CaMKII +/- mice, showing the increased excitability of granule cells, the decreased expression of calbindin, a marker of mature granule cells, and the increased expression of dopamine D1 receptors (Mol Brain I:6, 2008). We observed that chronic treatment of C57BL/6 mice with fluoxetine induced the expression of D1 receptors, but not other dopamine receptors, in granule cells of the dentate gyrus. The high expression of D1 receptors resulted in activation of cAMP/PKA signaling. In vivo microdialysis analysis revealed that the serotonin responses to a novel environment in the dentate gyrus were suppressed after chronic fluoxetine treatment due to high activity of D1 receptors. In behavioral studies, D1 receptor agonism was shown to enhance antidepressant action of fluoxetine in mice chronically subjected restraint stress. These findings suggest that the dopamine D1 receptor in the dentate gyrus plays a key role in the action of antidepressants, and is a therapeutic target of depression.

(COI: No)

Symposium 59

Physiological Model-Based Cardiovascular Diagnosis/Therapy

(March 23, 15:00～16:30, Room A)

S59-1

The monitoring and the clinical application of left ventricular arterial coupling (Ees/Ea)

Shigemi, Kenji[1]; Obata, Yurie[1]; Hayabuchi, Mitsuyo[1]; Takaku, Akiko[1]; Hamada, Toshihiko[2]; Okafuji, Kazuhiro[3]; Matsuoka, Satoshi[4] (*[1]Dept of Anesthesiol and Reanimatol, Fac of Med Sci, Univ of Fukui, Fukui, Japan; [2]Dept of Clin Lab, Univ of Fukui Hosp, Fukui, Japan; [3]Health Exam Ctr, Fukui-ken Saiseikai Hosp, Fukui, Japan; [4]Det of Integrative and Systems Physiol, Fac of Med Sci, Univ of Fukui, Fukui, Japan*)

The estimation of ventricular arterial coupling (Ees/Ea) is clinically useful for general anesthesia and critical care, since the cardiac performance, such as cardiac output and ejection fraction, depends on Ees/Ea, and the efficacy of energetic transfer from the heart to the artery is also related to Ees/Ea. We approximated the waveform of the left ventricular time varying elastance curve with two straight lines, and end-diastolic left ventricular pressure was supposed to be zero for estimating Ees/Ea with four non-invasive parameters: end-systolic arterial pressure (Pes), diastolic arterial pressure (Pad), pre-ejection period (PEP), and eject time (ET). In order to apply this estimation to clinical monitoring, we used mean arterial pressure (Pm) as Pes and cardio ankle vascular index (CAVI) as arterial elastance (Ea). Healthy individuals (2,675 males, 2,287 females), who visited the health examination center at Fukui-ken Saiseikai Hosp, were recruited to measure Pm, Pad, PEP, ET and CAVI with vascular screening system (VaSera VS-1500, Fukuda Denshi, Tokyo). Mean and SD values were as follows: Ees/Ea = 1.22 ± 0.61, Ees = 1.44 ± 0.58 (mmHg/ml). There are suggested that the estimated values vary widely and the accuracy of the values beyond the normal range are low.

(COI: No)

S59-2

Current clinical application and problems of central blood pressure estimation based on pulse waveform analyses

Miyashita, Hiroshi[1]; Katsuda, Shin-ichiro[2] (*[1]Jichi Med Univ Health Care Center, Tochigi, Japan; [2]Dept Cellular and Integrative Physiol, Fukushima Med Univ Sch Med, Fukushima, Japan*)

Central blood pressure (CBP) is increasingly recognized as an important cardiovascular risk marker in addition to peripheral blood pressure (BP). Because CBP cannot be directly measured noninvasively, it should be estimated from peripheral arterial pulses. Among various methods to estimate CBP, we have focused on 2 principal methods; 1) generalized pressure transfer function (GTF), 2) radial 2nd systolic peak pressure (SP2). Both methods are based on aorto-radial pressure wave transmission properties, which can be modeled as a single or parallel elastic tube. It was supported by a wave separation analysis of precise animal data of pressure wave transmission along the forelimb arteries. A frequency component analysis of simultaneous human central and radial artery pressure waveforms has shown that central augmentation peaks consisted predominantly of lower harmonic components, which might make SP2 approximate CBP. We have found equivalence of appropriately modified SP2- and GTF-based CBP estimates in humans and rabbits.

A comparison between Japanese and standard (Westerner's) GTFs suggested limitations of GTF. Though the difference between central and peripheral BPs is a perfectly dynamic phenomenon, absolute CBP levels largely depend on mean (static) BP levels. CBP estimation unexpectedly elucidated large inaccuracy of brachial cuff BP measurement that has been the only method to calibrate noninvasive CBP. The calibration issue is the biggest problem to be solved.

(COI: No)

S59-3

Directional sensitivity of the arterial baroreflex to pressure input and its implication in baroreflex activation therapy

Kawada, Toru; Shimizu, Shuji; Sugimachi, Masaru (*Dept Cardiovasc Dynamics, Natl Cereb Cardiovasc Ctr, Osaka, Japan*)

The arterial baroreflex is an important negative feedback system that stabilizes arterial pressure (AP) during daily activities. Although pulsatility of baroreceptor input pressure is known to affect the baroreflex response, whether the directional change in the pulsatility affects the baroreflex response remains unknown. The neural arc of the arterial baroreflex from pressure input to efferent sympathetic nerve activity (SNA) can be modeled by a derivative filter followed by a nonlinear sigmoidal component. This model predicts that a forward triangular wave (FTW) input (a ramp increase followed by an abrupt drop) is more effective to suppress SNA compared with a backward triangular wave (BTW) input (a ramp decrease followed by an abrupt increase). This prediction was examined in anesthetized Wistar-Kyoto rats. Carotid sinus baroreceptor regions were isolated from the systemic circulation, and carotid sinus pressure was changed according to FTW or BTW with a peak-to-peak amplitude of 40 mmHg at the same mean pressure. The BTW input increased the mean level of SNA compared with the FTW input. Mean AP was higher during the BTW input than during the FTW input. These results indicate that we may be able to suppress SNA and reduce AP more effectively using the FTW input. Recent investigations indicate that electrical stimulation of the carotid sinus baroreflex can be a therapeutic approach to drug resistant hypertension. Modulating the pulse train of the carotid sinus stimulation using FTW would be more effective for the treatment of hypertension.

(COI: No)

S59-4

Elevation of pulmonary input impedance in low frequency can worsen right ventricle-pulmonary artery coupling

Fuke, Soichiro; Kashihara, Yuya; Namba, Yusuke; Tanaka, Masamichi; Yumoto, Akihisa; Saito, Hironori; Sato, Tetsuya (*Dept Cardiology, Japanese Red Cross Okayama Hospital, Okayama, Japan*)

Background: The relationship between pressure reflection and right ventricular (RV) function is not well known.

Methods: Nineteen patients suspected of having pulmonary hypertension (PH) were enrolled. Patients with a mean pulmonary artery pressure (PAP) of >20 mmHg were included in the PH group (n=9; mean PAP, 28 ± 7 mmHg), and others were included in the normal group (n=10; mean PAP, 13 ± 2 mmHg). The central aortic pressure was recorded in 10 patients. PAP and flow velocity by using ultrasound was simultaneously recorded. Additionally, RV pressure and flow velocity were recorded. Aortic flow velocity was estimated with the triangular wave. The characteristic impedance (Zc) was calculated in a time-domain manner. The maximal RV hydromotive pressure was estimated by using an extrapolated sin curve fitted to the isovolemic pressure, and Ees/Ea was then calculated using these values.

Results: |Z1|/Zc was smaller in the normal group than in the central aorta (1.19 ± 0.45 vs. 1.91 ± 0.68, p=0.012). |Z1|/Zc and |Z2|/Zc were larger in the PH group than in the normal group (2.24 ± 1.09 vs. 1.19 ± 0.45, p=0.012; 1.48 ± 0.72 vs. 0.89 ± 0.31, p=0.028, respectively). Augmentation index was elevated and Ees/Ea was decreased to a greater extent in the PH group than in the normal group (37.7 ± 23.8% vs. 8.8 ± 11.2%, p=0.003; 0.65 ± 0.42 vs. 1.84 ± 0.69, p<0.001, respectively).

Conclusion: In PH patients, elevation of pulmonary input impedance in low frequency and pressure reflection may cause RV-PA decoupling.

(COI: No)

S59-5

Intraoperative transit time flowmetry: How can physiology and anatomy predict clinical outcomes after CABG ?

Une, Dai[1,2]; Kikuchi, Keita[1]; Endo, Yoshiki[1]; Matsuyama, Takayoshi[1]; Fukada, Yasuhisa[1]; Kurata, Atsushi[1] (*[1]Div Cardiovasc Surg, Yamato Seiwa Hosp, Kanagawa, Japan; [2]Div Cardiac Surg, University of Ottawa Heart Institute, Ottawa, Canada*)

Background: Intraoperative transit time flowmetry is helpful in detecting technical errors such as bypass twist and stenosed anastomosis by measuring the volume of bypass flow and flow patterns. Also, it was reported that lower mean graft flow was associated with mid-term graft failure. We evaluated optimal cut-off values of graft flow for 1-year graft failure, and its risk factors.

Methods: Sixty-five saphenous vein grafts were examined with transit time flowmeter and fluorescent angiography during isolated coronary artery bypass grafting. Then, all the grafts were evaluated with angiography 1 year after operation. Receiver operating characteristics analysis was performed to decide the best cut-off value.

Results: The 1-year patency of vein grafts was 65% although all the grafts were patent in operative room. The best cut-off value of mean graft flow was 31 ml/hr, and this was a significant risk factor. However, its sensitivity and specificity to detect 1-year graft failure was approximately 65%. There was no other predictor.

Conclusions: The diagnostic ability of transit time flowmetry is not good enough although it was the only significant predictor of 1-year graft failure. When graft flow volume is same, larger grafts have less shear stress with slower blood flow, as a result, more hyperplasia. Probably, surgeon may be able to predict 1-year to mid-term graft failure more accurately by considering the size of vein graft.

(COI: No)

S59-6

Total unloading of left ventricle by circulatory assist device minimizes the infract size in ischemia-reperfusion injury

Saku, Keita; Kakino, Takamori; Sunagawa, Kenji (*Dept Cardiovasc Med, Kyushu Univ, FUK, JPN*)

Backgrounds: Although LVAD has been extensively used both in acute and chronic heart diseases, how LVAD impacts on cardiac energetics as well as mechanics remains poorly understood. The pressure-volume area (PVA) of the left ventricle (LV) has been shown to be tightly coupled with myocardial oxygen consumption (MVO2). Theoretical analysis indicates that, the partial LVAD support (PARTIAL) where LV remains ejecting, reduces LV preload while increases afterload, and thus does not decrease much the PVA. In contrast, the total LVAD support (TOTAL) where LV no longer ejects, markedly decreases PVA, thereby MVO2. We hypothesized PARTIAL and TOTAL unloading would have major differences in the infract size in ischemia-reperfusion injury.
Methods: In 12 anesthetized dogs, we created ischemia by occluding major branches of the left anterior descending coronary artery for 90 min, reperfused for the following 300 min. We compared the infract size (normalized by the risk area) among 3 groups, no support (CONT), PARTIAL (LV output=LVAD flow) and TOTAL (no LV output).
Results: Mean arterial pressure did not differ among 3 groups, while left atrial pressure significantly reduced in TOTAL (CONT: 11±5, PARTIAL: 8±4, and TOTAL: -1±3 mmHg, $p < 0.01$). LVAD significantly reduced the infarct size (CONT: 40±3.2, PARTIAL: 27.6±5.8, and TOTAL: 5.0±1.6%, $p < 0.01$). The reduction of infarct size was by far lager in TOTAL (-88%) than in PARTIAL (-31%). **Conclusions:** Total unloading of LVAD minimizes the metabolic demand and maximize the beneficial impact on ischemia-reperfusion injury.

(COI: No)

Symposium 60

Integrated approaches to understand the pathophysiology of dystonia and involuntary movement

(March 23, 15:00～16:30, Room C)

S60-1

Dystonia -definition and multifaced phenotype-

Hasegawa, Kazuko (*Dept Neurolgy, National Hospital Organization, Sagamihara National Hospital*)

Dystonia is one of the common neurodegenerative disorders, however, clinical diagnosis is difficult. Dystonia is currently defined as a neurologic syndrome characterized by involuntary, sustained, patterned, and often repetitive muscle contractions of opposing muscles, causing twisting movements or abnormal postures. This definition may be difficult or cumbersome to apply for neurologists faced with patients in the clinic, causing underdiagnose of dystonia in clinical practice. Numerous causes evoke dystonia exactly, so that classification of dystonia is based on etiological role, such as genetic, primary, according to neurodegenerative disorders. I will try to present here many face of dystonia by video, and its classification.

(COI: No)

S60-2

Dystonia model mouse deficient of Na-pump alpha3 subunit gene

Kawakami, Kiyoshi[1]; Ikeda, Keiko[2]; Chiken, Satomi[3]; Sugimoto, Hiroki[1]; Nambu, Atsushi[3] (*[1]Division of Biology, Center for Molecular Medicine, Jichi Medical University, Tochigi, Japan; [2]Biology, Hyogo Medical College, Hyogo, Japan; [3]Division of System Neurophysiology, National Institute for Physiological Sciences*)

ATP1A3 is the causative gene for rapid-onset dystonia parkinsonism (RDP) and alternating hemiplegia of childhood (AHC). Many of the mutations found in patients are substitution mutations and most of the positions of mutations are distinct between RDP and AHC. The mutations are mostly loss of function mutations and lead to decreased activity of Na, K-ATPase. We established a *Atp1a3* gene deficient mice and performed behavioural and electrophysiological analyses related to symptoms of RDP and AHC. $Atp1a3^{+/-}$ showed increased symptoms of dystonia when being administered kainate into cerebellum (Ikeda et al J. Physiol. 2013). $Atp1a3^{+/-}$ showed shorter stride length in chronically-stressed condition. We next evaluated neuronal activity of basal ganglia under non-anesthesia condition. The spontaneous discharge rate of GPi neurons were significantly lower in $Atp1a3^{+/-}$ compared with wild-type. The triphasic response pattern evoked by cortical stimulation in GPi and GPe in the wild-type mice was altered in $Atp1a3^{+/-}$. The changes of the firing pattern are similar to those observed in DYT1 dystonia (Chiken et al . J. Neurosci 2008). The inhibitory neurotransmission from molecular-layer interneuron to Purkinje cells in the developing cerebellum was enhanced. The contents of dopamine and its metabolite were reduced in $Atp1a3^{+/-}$. These observations suggest the usefulness of $Atp1a3^{+/-}$ as a model of RDP.

(COI: No)

S60-3

Mouse model of dystonia with sensory neuropathy

Takebayashi, Hirohide (*Grad. Sch. Med. Dent. Sci., Niigata Univ., Niigata, Japan*)

Dystonia is a disorder characterized by involuntary muscle contractions that cause slow repetitive movements or abnormal postures. Dystonia musculorum (*dt*) mouse is an inherited dystonia model mouse with sensory neuropathy. *Dystonin* (*Dst*) is a causative gene for *dt* mice, which encodes for a cytoskeletal linker protein. We had generated a novel *Dst* gene trap (Dst^{Gt}) mice, in which actin-binding domain-containing isoforms are disrupted. Homozygous Dst^{Gt} mice showed typical *dt* phenotypes with progressive neurological symptoms: severe motor disorders in their limbs and twisted postures. Electomyogram showed abnormal co-contractions of agonist and antagonist muscle in the homozygotes. In histological analyses, abnormal neurofilament accumulation was observed in both peripheral nervous system and central nervous system. Since this Dst^{Gt} allele is a multipurpose conditional allele, we will perform conditional knockout and rescue experiments to investigate neuronal circuits responsible for dystonia phenotype.

(COI: No)

S60-4

Dystonia, basal ganglia and cerebellum

Nambu, Atsushi (*Div System Neurophysiol, Natl Inst Physiol Sci, Okazaki, Japan*)

Dystonia is a neurological disorder characterized by sustained or repetitive involuntary muscle contractions and abnormal postures. Reduced spontaneous activity with bursts and pauses has been reported in both internal (GPi) and external (GPe) segments of the globus pallidus in dystonia patients, especially in generalized dystonia. Similar activity changes were reported in dystonia animal models as well. In addition, deep brain stimulation, i.e., high-frequency stimulation in the GPi ameliorates dystonic symptoms. These observations suggest that the origin of dystonia is in the basal ganglia, and indicate the following pathophysiology: In normal state, GPi outputs suppress unnecessary movements, whereas in dystonia, reduced GPi outputs cause increased thalamic and cortical activity, resulting in involuntary movements. On the other hand, a number of dystonia animal models have been reported to be of cerebellar origin. For example, Wriggle Mouse Sagami, which exhibits coactivation of agonist and antagonist muscles and abnormal postures, showed decreased spontaneous activity of cerebellar Purkinje cells, with no significant activity changes in the basal ganglia. Another example is ouabain injection into the cerebellar nuclei, which was reported to induce dystonic symptoms through the cerebello-thalamo-striatal pathway. In this symposium, I would like to discuss the contribution of the basal ganglia and cerebellum to pathophysiology of dystonia.

(COI: No)

S60-5

Electrophysiological hallmarks for dystonia

Darbin, Olivier E (*Dept Neurology, Univ. South Alabama, Mobile, USA*)

Dystonia is a movement disorder with sustained and involuntary muscle contractions causing abnormal postures, twisting and repetitive movements. Dystonia can be a primary condition or secondary to brain trauma, infection, poisoning or unusual physical activity. Treatments include therapeutics for peripheral targets to reduce muscular contraction (anticholinergic drugs, butolin toxin or muscle relaxant) or central targets to 'normalize' the activity in motor circuitry (deep brain stimulation, baclofen). In absence of gross neuro-anatomical hallmarks, the physiopathology of dystonia remains poorly understood and based from the analyses of either electro-encephalograph, neuro-signals collected per -operative during procedure for deep brain implantation and, more marginally, from animal models.Pathological findings include abnormalities at the levels of the cortices, brainstem and basal ganglia in motor and sensory territories. Physiological abnormalities include topographic des-organization in the cortico-basal ganglia loops and impaired sensorimotor integration. At the level of the basal ganglia, and the globus pallidus specifically, there are a decreased firing activity, slower/weaker oscillations and increased occurrences in pauses and bursts. Data from primate suggests also that striatal gabaergic control may contribute to dystonic-like movement and/or be a therapeutic target.However, integrated interpretation of these data has not been reached. The causal relationship of these abnormalities to dystonic movements remain unestablished. The lack of 'gold standard' animal model is an obstacle to identify the underlying mechanisms of dystonia.

(COI: No)

Symposium 61

Morphological and functional mechanisms and their dynamics in the multimodality of inhibitory neural system

(March 23, 15:00～16:30, Room D)

S61-1

Local Impermeant Anions Establish the Neuronal Chloride Concentration

Egawa, Kiyoshi[1,2] ([1]*Dept Pediatrics, Hokkaido Univ. Hospital;* [2]*Mass. General Hospital, Boston, U.S.A.*)

Neuronal intracellular chloride concentration $[Cl^-]_i$ is an important determinant of g-aminobutyric acid type A ($GABA_A$) receptor mediated inhibition and cytoplasmic volume regulation. Equilibrative cation-chloride cotransporters (CCCs) move Cl^- across the membrane, but accumulating evidence suggests factors other than the bulk concentrations of transported ions determine $[Cl^-]_i$. To investigate regulatory mechanisms of $[Cl^-]_i$, we measured neuronal $[Cl^-]_i$ in murine brain slice preparations expressing the transgenic fluorophore Clomeleon. Main results are as following. 1) somatic $[Cl^-]_i$ are negatively correlated with SYTO staining intensity, which corresponds to cytoplasmic nucleic acid proteins. 2) $[Cl^-]_i$are also inversely correlated with the amount of negatively charged extracellular matrix evaluated by alcian blue staining. 3) $[Cl^-]_i$ increased when a part of $[Cl^-]_i$, was released by gluconate or pyruvate. 4) Chondroitinase ABC, an inhibitor of extracellular matrix, also increased $[Cl^-]_i$. CCC inhibition had modest effects on $[Cl^-]_i$ and neuronal volume, but substantial changes were produced by alterations of the balance between $[A^-]_i$ and $[A^-]_o$. Therefore, CCCs are important elements of Cl^- homeostasis, but local impermeant anions determine the homeostatic set point for $[Cl^-]_i$ and hence, neuronal volume and the polarity of local $GABA_A$ receptor signaling.

(COI: No)

S61-2

Functional regulation of neuronal K^+-Cl^- cotransporter KCC2

Watanabe, Miho[1]; Iwata, Satomi[1]; Furukawa, Tomonori[2]; Kumada, Tatsuro[3]; Uchida, Taku[4]; Hirose, Shinichi[4,5]; Fukuda, Atsuo[1] ([1]*Dept Neurophysiol, Hamamatsu Univ Sch Med, Hamamatsu, Japan;* [2]*Dept Neurophysiol, Hirosaki Univ, Hirosaki, Japan;* [3]*Dept Occupational, Tokoha Univ, Hamamatsu, Japan;* [4]*Inst Mol Pathomechanisms Epilepsy, Fukuoka Univ, Fukuoka, Japan;* [5]*Dept Pediatrics, Fukuoka Univ, Fukuoka, Japan*)

The neuronal K^+-Cl^- cotransporter (KCC2) is a membrane transport protein that extrudes Cl^- from neurons and helps maintain low intracellular $[Cl^-]$ and hyperpolarizing GABAergic synaptic potentials. Several neurological disorders are associated with decreases in the Cl^- extrusion capacity of KCC2 that result in increase of $[Cl^-]_i$ and subsequent hyperexcitability of neuronal networks. Despite the importance for plasticity of inhibitory transmission, less is known about the regulation of the intrinsic KCC2 activity. KCC2 consists of 12 transmembrane domains (TMDs) and flanked by two intracellular termini. Recently several groups have identified in the KCC2 molecule different regions and amino acid residues that regulate the KCC2 transport activity or its cell surface stability. Here, we showed novel regulatory site of KCC2 activity. Mutation of phenylalanine residue (F571I) located inside 10th putative TMD inhibited ion-transport activity. In contrast, mutation of leucine residue (L907V) located in C-terminus resulted in up-regulation of transport activity. But mutation of proline residue (P384A) located large extracellular loop between TMDs 5th and 6th or arginine residue (R592Q) located inside 11th putative TMD did not affect transport activity. These results suggest that F571 and L907 are critically residues involved in KCC2 activity.

(COI: No)

S61-3

Taurine regulates the intrinsic properties of the neural progenitors as a ligand for GABAA receptors in the mouse developing neocortex

Tochitani, Shiro (*Res. Cent. Child Mental Development, Univ. Fukui, Fukui, Japan*)

Precise temporal regulation of the intrinsic properties of neural stem cells (NSCs) to produce the diverse type of neurons and glial cells underlies the formation of the complex structure of central nervous system. We obtained evidences that $GABA_A$ receptors participate in this regulatory machinery. Fetal exposure to positive allosteric modulators for $GABA_A$ receptors at E10-11 accelerated the transition from neuroepithelial cells to BLBP-positive radial glia, resulting in the changes in the timing of neurogenesis. Exposure to $GABA_A$ positive modulators at E10-E12 enhanced the differentiation into Satb2-positive upper-layer neurons and suppressed the differentiation into Tbr1-positive deep-layer neurons. Exposure to $GABA_A$ antagonists at the same embryonic stages caused the opposite effects in terms of these four properties of neural progenitors. Both GABA and taurine are known as endogenous ligands for $GABA_A$ receptors. Immunohistochemical analyses and HPLC quantification showed that taurine is dominant in quantity among the endogenous ligands for $GABA_A$ receptors in the developing cortex before E14. The E12 taurine transporter-knockout mouse cortices exhibited the phenotypes that well resembled those observed in the embryos exposed to $GABA_A$ antagonists. These results show that $GABA_A$ receptor activation principally by taurine plays a crucial role in the regulation of the properties of NPCs in the early phase of cortical development.

(COI: No)

S61-4

Characteristic development of GABAergic transmission in the mouse spinal cord

Kim, Jeongtae; Kosaka, Yoshinori; Takayama, Chitoshi (*Grad.Sch.Med., Ryukyu Univ., Okinawa, Japan*)

In the mammalian central nervous system, gamma-amino butyric acid (GABA) is a predominant inhibitory neurotransmitter, whereas it acts as an excitatory transmitter in the immature CNS, and may be involved in morphogenesis. We have investigated the ontogeny of the GABAergic transmission by immunohistochemistry for glutamic acid decarboxylase (GAD), GABA transporters (GATs), vesicular GABA transporter (VGAT), and potassium chloride cotransporter2 (KCC2) in the mouse cervical spinal cord. In this session, we present the developmental changes in GABAergic transmission. (1) Before synapse formation, GABA may be extrasynaptically released by non-exocytotic system, and be transported into the processes of radial glia or immature astrocytes by GAT-3. (2) In the ventral horn, GABAergic neurons appear on embryonic day 12 (E12), synapses are formed after E13, and increased in number after E15. Synaptically released GABA was removed by only GAT-3 on the processes of astrocytes. (3) In the dorsal horn, GABAergic neurons are localized after E13, synapses are formed after E17, and increased in number after birth. Synaptically released GABA is removed by GAT-1 into presynapse and GAT-3 into processes of astrocytes. (4) GABA may act as an excitatory transmitter for several days before GABAergic synapse were formed in the embryonic spinal cord. (5) During development, GABAergic synapses are decreased in the ventral horn, whereas glycinergic synapses are increased.

(COI: No)

Symposium 62

Functional roles of monoaminergic/cholinergic neurotransmitters in higher order behaviors

(March 23, 15:00～16:30, Room E)

S62-1

Functional roles of cortical cholinergic modulation in visual contrast detection behavior

Soma, Shogo (*Grad Sch Med, Osaka Univ, Osaka, Japan*)

The cholinergic neurons originating in the nucleus basalis of the basal forebrain (BF) innervate the entire cortical mantle, releasing acetylcholine (ACh) context-dependently to optimize various cognitive processes such as sensation, attention, learning and memory, and decision making. However, it remains unclear how the optimization of cortical processing is realized. One target of the cholinergic projections is the primary visual cortex (V1), and ACh regulates the V1 neuron's activities in many species. In this talk, I focus on the cholinergic modulation of visual information processing at the neuronal and behavior levels. Recently, we extracellularly recorded visual responses to drifting sinusoidal grating stimuli from V1 of anesthetized animals (monkey and rat) and tested the effects of ACh applied locally by microiontophoresis or widely by topical administration. ACh modulated the visual responses in V1 by controlling the response gain and improving signal-to-noise ratio. To examine whether such cholinergic regulations of visual information processing contributes to the visual performance in behaving animals, we trained rats to detect visual stimulus in a two-alternative forced-choice task combined with a staircase method. We found that contrast detectability was improved by the systemic injection of donepezil, a cholinesterase inhibitor, and impaired by the immunolesion of BF cholinergic neurons by 192 IgG-saporin. Therefore, ACh endogenously released in cognitive behavior controls the contrast detectability by modulating cortical visual information processing to meet the purposes of behavioral context.

(COI: No)

S62-2

Imaging and implications of dopaminergic neurons for movement disorder: "opposite sides of the same coin" in Parkinson's disease

Matsuda, Wakoto[1]; Furuta, Takahiro[2]; Nakamura, Kouich C[2]; Hioki, Hiroyuki[2]; Kaneko, Takeshi[2] (*[1]LIMS, Kyoto Univ, Kyoto, Japan; [2]Morphol Brain Sci, Grad Sch Med, Kyoto Univ, Kyoto, Japan*)

The recent developments in brain imaging have offered new insights into the morphology of dopaminergic (DA) neurons. In this symposium, we describe these new morphological measurement techniques and how they contribute to our understanding of movement disorder, especially of Parkinson's disease (PD). We present novel imaging techniques using Sindbis virus vectors that coded membrane-targeted green fluorescent protein (GFP) that reveal important new structural information concerning DA neurons. Detail morphological images of DA neurons derived from this new approach are used to elucidate the role of DA neurons in PD. First, we point out how the new images reveal how DA neurons have a massive axonal arborization in the striatum. This arborization is on a scale not previously known, and of a form that implies both a particular vulnerability and a redundancy in DA neurons. Second, we describe how the imaging results indicate that DA neurons innervate both the striosome and the matrix compartments of the striatum. This dual innervation has implications for reinforcement learning in the basal ganglia and for how normal behavior is driven and how it may be disrupted by Levodopa PD therapies. We conclude with a summary of how these results contribute to our understanding of PD.

(COI: No)

S62-3

Implications for the monoaminergic/cholinergic basis of impulsive behavior

Yoshioka, Mitsuhiro; Kimura, Iku Tsutui; Ohmura, Yu (*Dept Neuropharmacol, Grad Sch Med, Hokkaido Univ, Sapporo, Japan*)

Impulsive actions are often viewed as everyday normal behavior; however, excessive levels of impulsivity are associated with several psychiatric disorders, such as attention-deficit/hyperactivity disorder, schizophrenia, and borderline personality disorder. Moreover, it could be a risk factor for drug addiction, criminal involvement, and suicide. We focused on the three-choice serial reaction time task, which is one of the most appropriate and simple rodent model of impulsive-like action and is based on the human continuous performance test. We examined implications for the monoaminergic/cholinergic basis of impulsive behavior using pharmacological intervention such as administration of not only monoamine-related but also nicotinic acetylcholine receptor (nAChR)-related drugs. We have elucidated the mechanism of action in some of these drugs. For example, we demonstrated that a milnacipran, a serotonin/noradrenaline reuptake inhibitor, enhanced the control of impulsive action by activating dopamine D1-like receptors in the infralimbic cortex (IL), and that intra-IL infusion of a selective $\alpha 4\beta 2$ nAChR antagonist dose-dependently blocked nicotine-induced impulsive-like action.In this symposium, we introduce recent advances in this field and describe the role of not only nAChR-related but also monoamine-related brain mechanisms in modulating impulsive behavior. We also suggest several potential therapeutic drugs to address these mechanisms in impulsivity-related disorders and explore future directions to develop anti-impulsive drugs.

(COI: No)

S62-4

Serotonergic involvements of sociality and mental disorder: Molecular imaging study by PET in non-human primates

Onoe, Hirotaka (*Imaging Function Group, Cent Life Sci Tech, RIKEN, Kobe, Hyogo, Japan*)

Serotonin is involved in regulating emotional and social behaviors, and also formation of social behavioral traits in humans and other primates. It also appears to be one of the major players in mood and mental disorders such as the major depression. But exactly how remains an open question. The availability of positron emission tomography (PET) for human and nonhuman primates has enabled examination of the in vivo functions of specific neurotransmitter systems underlying social behavior. We established a PET imaging system for conscious macaque monkeys and also common marmosets, a small primate species noted for its high social tolerance and cooperative sociality. We used this method to examine the dopaminergic and serotonergic systems in the brain using [^{11}C]DASB and [^{11}C]AZ10419369 , which are highly selective to serotonin transporter (SERT) and serotonin 1B (5-HT1B) receptor, respectively. Using parametric images of binding potential (BP) values and behavioral scores determined by social test in marmosets, we processed on the statistical mapping to identify brain areas of which BP values of SERT which are tightly associated with social behavior. We also investigate recently that pharmacological anti-depressive action of ketamine on 5-HT1B receptor. Results demonstrate that molecular imaging of the brain can provide valuable information for understanding the neural bases of personality and anti-depressive action in nonhuman primates. All procedures of this study were approved by the Animal Care and Use Committee of Kobe Institute in Riken

(COI: No)

Symposium 63

Frontiers in sleep research

(March 23, 15:00～16:30, Room F)

S63-1

Spacio-temoporal cellular circuit profiling for the organism-level systems biology

Susaki, Etsuo A[1,2] (*[1]Dept of Systems Pharm., Grad. Sch. Med, Univ Tokyo, Tokyo, Japan; [2]Lab. for Synthetic Biology, RIKEN QBiC, Kobe, Japan*)

Circuit-level identification and analysis of neural networks in the brain will require the development of whole-brain imaging with single-cell resolution. To this end, we performed comprehensive chemical screening to develop a whole-brain clearing and imaging method, termed CUBIC (Clear, Unobstructed Brain Imaging Cocktails and Computational analysis). CUBIC is a simple and efficient method involving the immersion of brain samples in a chemical mixture which enables rapid whole-brain imaging with single-photon excitation microscopy. CUBIC is applicable to multi-color imaging of fluorescent proteins or immunostained samples in adult brains, and is scalable from a primate brain to subcellular structures. We also developed a whole-brain cell-nuclear counterstaining protocol and a computational image analysis pipeline which, together with CUBIC reagents, enable the visualization and quantification of neural activities induced by environmental stimulation. CUBIC enables time-course expression profiling of whole adult brains with single-cell resolution. We are also developing high-throughput, next generation-type mouse genetics by using "ES-mouse" technology. The combinations of CUBIC and ES-mouse genetics will provide basics for realizing the organism-level systems biology.

(COI: No)

S63-2

The role of PPARs and ketone body metabolism in the regulation of sleep homeostasis

Chikahisa, Sachiko; Sei, Hiroyoshi (*Dept Integ Physiol, Tokushima Univ, Tokushima, Japan*)

Sleep regulations are associated with energy metabolism. Sleep restriction leads to metabolic disorders such as obesity and diabetes, but the mechanisms that underlie its effects remain unclear. We have recently found that peroxisome proliferator-activated receptors (PPARs) are involved in the regulation of sleep homeostasis. PPARs are transcriptional factors belonging to the nuclear receptor family which relate to the regulation of glucose and lipid metabolism. Chronic treatment of bezafibrate, a PPARs agonist, advanced wake/sleep pattern, and enhanced the slow-wave activity (SWA) in non-rapid eye movement (NREM) sleep in mice. Plasma concentration of ketone bodies acetoacetate (AcAc) and β-hydroxybutyrate (BHB) were also affected by bezafibrate. Bezafibrate-treated mice showed a marked increase in plasma AcAc and decrease in BHB. Ketogenesis is modulated by the activity of PPARα, one of the three PPARs isotypes, under the condition of low glucose availability. To investigate the specific effects of ketone bodies on sleep homeostasis, plasma concentration of ketone bodies were measured after sleep deprivation for 6 hours. Sleep deprivation increased plasma ketone bodies and increased mRNA expression of PPARα and mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase 2 (Hmgcs2), a rate-limiting enzymes of ketogenesis, in the hypothalamus and cortex. In addition, central injection of AcAc, but not BHB, increased SWA during NREM sleep and suppressed glutamate release. Our results suggest that central PPARα and metabolism of ketone bodies (especially AcAc) play a role in the regulation of sleep homeostasis.

(COI: No)

S63-3

Novel classification and function of rat thalamic neurons on the basis of the single-cell labeling studies

Kuramoto, Eriko (*Dept. Oral Anat. and Cell Biol., Grad. Sch. Medical and Dental Sci., Kagoshima Univ., Kagoshima, Japan*)

The thalamus not only acts as a relay between subcortical areas and the cerebral cortex, but also appear to play an important role in regulating arousal and attention. Recently it has been proposed that thalamic neurons are divided into core and matrix neurons: core neurons are found principally in sensory thalamic nuclei, and project to middle layers of the cortex in an area-specific manner; matrix neurons project to superficial layers of cortex over wide areas (Jones 1998). In the present studies, we analyzed axonal arborization of the rat thalamic neurons project to the frontal cortex by a single neuron labeling method with a Sindbis viral vector, and tried to apply the core-matrix classification to our results. The mediodorsal (MD) and caudal part of the ventral anterior-ventral lateral nuclei (VA-VLc) neurons mainly projected to middle layers like core neurons, however, the cortical fields covered by axon fibers of single neurons are not restricted in a cortical area. Thus, there might be at least two subtypes in the core neurons, focal- and diffuse-type core neurons. In contrast, ventromedial (VM) and rostral VA-VL (VA-VLr) neurons sent their axon fibers predominantly to superficial layers of widespread cortical areas, indicating that these neurons are classified into the matrix type. The result suggests that, even when a small number of the VM or VA-VLr neurons are activated, many pyramidal neurons in widespread cortical areas would be activated through the apical dendrites, and may thus be associated with arousal or attentional mechanism.

(COI: No)

S63-4

Optical dissection of the sleeping cortex

Kanda, Takeshi[1]; Tsujino, Natsuko[1]; Ishii, Ryo[1]; Yanagisawa, Masahi[1,2] (*[1]International Institute for Integrative Sleep Medicine, Univ Tsukuba, Ibaraki, Japan; [2]University of Texas Southwestern Medical Center, TX, USA*)

Sleep is the neurophysiological process of the brain, regulated by the brain, and required for the brain. Despite of its importance, the physiological nature of sleep has not been well explored. In mammals, cortex is highly susceptible to sleep. In fact, electroencephalography (EEG)-based criteria is generally admitted to determine the states of sleep and wakefulness. During NREM sleep, EEG is dominated by slow-wave activity. The slow-wave activity in the EEG and the underlying variations in local field potentials (LFP) occur regionally on a macro scale. However EEG, LFP and unit recordings cannot estimate the precise location of recording areas and cells on a microscopic scale. To explore the spatio-temporal pattern of individual neuron activity in the cortex during sleep and wakefulness, using two-photon microscopy, we performed Ca^{2+} imaging in the layer 2/3 of primary motor cortex of naturally sleeping and awake mice. When mice were awake and running, Ca^{2+} dynamics was highly active and the temporal patterns were not coordinated between neurons. The transition to synchronized state of Ca^{2+} activity was observed when mice were falling asleep. The highest synchronization appeared for typical NREM sleep. The synchronicity of spontaneous Ca^{2+} signals among neurons did not depend on the distance between neurons on micrometer scale. Our results, taken together with other studies, suggest that cortical neuron activity synchronize temporally during sleep and that the synchronization is regional but not completely local.

(COI: No)

S63-5

Forward genetic approach toward the elucidation of sleep/wakefulness regulation

Funato, Hiromasa[1,2] (*[1]Univ Tsukuba WPI-IIIS, Ibaraki, Japan; [2]Toho Univ, Med, Sch, Tokyo, Japan*)

Although sleep is a ubiquitous animal behavior, the molecular mechanism of sleep homeostasis remains unknown. We performed high-throughput screening of ENU-mutagenized mice in order to identify genes regulating sleep/wake behavior. We have so far analyzed EEG/EMG data of more than 6,000 mutagenized male mice. We established several pedigrees showing heritable sleep/wakefulness abnormalities. Among them, the Sleepy mutant pedigree shows 30% reduction in 24-h wake time. To map a chromosomal region responsible for the sleep phenotype of Sleepy mutant mice, we performed a linkage analysis in N2 mice, obtained by backcrossing the mutagenized founder C57BL/6J male to C57BL/6N female mice for two generations. The analysis revealed a single peak with a LOD score of more than 20. Whole exome sequencing of mutants and wild-type littermates from the Sleepy pedigree identified a nucleotide change specific to Sleepy mutant mice within the mapped chromosomal region. The single nucleotide substitution abrogates a splice donor site of the gene that we termed Sleepy. RT-PCR analysis of the brain and liver mRNA found a short variant of Sleepy mRNA specific to Sleepy mutant mice. Functional analyses of the Sleepy gene are now underway.

(COI: No)

S63-6

Neural mechanisms for inducing fluctuations of autonomic nervous system during REM sleep

Koyama, Yoshimasa; Nishimura, Kunihiro; Haruyama, Naoto; Aoyagi, Takafumi
(*Dept Sci and Technol Fukushima Univ. Fukushima Japan*)

REM sleep is characterized by EEG desynchronization and muscular atonia. In addition to these tonic events, several phasic events such as rapid eye movement (REM) or fluctuation of autonomic nervous system occur during REM sleep. Blood pressure also displays large fluctuation during REM sleep. However, the mechanisms controlling it remains unknown. In the mesopontine tegmental area including laterodorsal/pedunculotegmental nuclei (LDT/PPT), some neurons become contentiously active prior to and during REM sleep and are considered to generate REM sleep, while considerable number of neurons showed phasic firing during REM sleep. About 44 % of neurons recorded in and around the LDT had a correlation with blood pressure fluctuation during REM sleep. Emotional changes during waking cause changes in autonomic nervous system including blood pressure. The amygdala is considered to be a center of emotion during waking. Since it is active during REM sleep, amygdala are hypothesized to be involved in blood pressure fluctuation during REM sleep. More than half of the amygdala neurons became active during REM sleep, but increase in firing occurred after the onset of REM sleep and the firing are mostly phasic. In about 39% of the amygdala neurons, their firing had a correlation with blood pressure during REM sleep. These results indicate that brainstem REM generation area and the amygdala are deeply involved in blood pressure fluctuation during REM sleep. Functional relations between two areas are discussed

(COI: No)

Symposium 64

Anatomical and physiological perspective of brain environment

(March 23, 15:00～16:30, Room H)

S64-1

Novel subtypes of astrocytes regulate neuronal excitability via release of gliotransmitters

Miwa, Hideki; Shibasaki, Koji (*Gunma Univ. Grad. Sch.of Med., Gunma, Japan*)

Astrocytes play active roles in the regulation of synaptic transmission. Neuronal excitation can evoke Ca^{2+} transients in astrocytes, and these Ca^{2+} transients can modulate neuronal excitability. While only a subset of astrocytes appears to communicate with neurons, the types of astrocytes that can regulate neuronal excitability are poorly characterized. We found that 20% of astrocytes in the brain express transient receptor potential vanilloid 4 (TRPV4), indicating that astrocytic subtypes can be classified on the basis of their expression patterns. When $TRPV4^+$ astrocytes are activated by ligands such as arachidonic acid, the activation propagates to neighboring astrocytes through gap junctions and by ATP release from the $TRPV4^+$ astrocytes. Following activation, both $TRPV4^+$ and $TRPV4^-$ astrocytes release glutamate, which acts as an excitatory gliotransmitter to increase synaptic transmission through group 1 mGluR. Our results indicate that $TRPV4^+$ astrocytes constitute a novel subtype of the population and are solely responsible for initiating excitatory gliotransmitter release to enhance synaptic transmission. In addition to $TRPV4^+$ subtype astrocytes, we identified that another unique subtype of astrocytes, which can release an inhibitory gliotransmitter. For the first time, we revealed that astrocytes transduce pre-synaptic signals to other post-synaptic signals. Our finding indicates that astrocytes are an important member of neural network. Especially, we identify the neuron-astrocyte-neuron, a sort of the triangle cell-cell communications.

(COI: No)

S64-2

Pathophysiological roles of TRPM2 expressed in the monocytic lineage cells

Kaneko, Shuji[1]; Shirakawa, Hisashi[1]; Nakagawa, Takayuki[2] ([1]*Dept Mol Pharmacol, Grad Sch Pharm Sci, Kyoto Univ, Kyoto, Japan;* [2]*Dept Pharm, Kyoto Univ Hosp, Kyoto, Japan*)

Several lines of evidence suggest that neuroinflammation mediated by the interaction between immune cells and neurons plays an important role in the pathogenesis of neuropathic pain. Transient receptor potential melastatin 2 (TRPM2) is a nonselective Ca^{2+}-permeable cation channel that acts as a sensor for reactive oxygen species. Using TRPM2-knockout mice, we examined the roles of TRPM2 expressed on immune and glial cells in neuropathic pain. TRPM2 deficiency attenuated pain behaviors in various kinds of inflammatory and neuropathic pain, but not in nociceptive pain models. In peripheral nerve injury-induced neuropathic pain models, TRPM2 deficiency diminished infiltration of neutrophils mediated through CXCL2 production from macrophages around the injured peripheral nerve and activation of spinal microglia. Also, in an in-vitro study with mouse primary microglia, lipopolysaccharide in the presence of interferon-γ activated TRPM2-mediated Ca^{2+} signaling and increased the downstream p38 MAPK and JNK signaling that resulted in elevated NO and CXCL2 production. Analysis of wildtype x TRPM2-knockout mixed-bone marrow chimeric mice revealed that TRPM2 plays an important role in the infiltration of peripheral immune cells, particularly macrophages, into the spinal cord, rather than into the injured nerves. The spinal infiltration of macrophages mediated by TRPM2 may contribute to the pathogenesis of neuropathic pain. We also discuss about the role of microglial TRPM2 in the aggravation of ischemic brain damage.

(COI: No)

S64-3

The roles of autophagy and lysosomal proteolysis for the maintenance of the normal environment in neuronal axons: lessons from the comparative analyses of cathepsin D- and Atg7-deficient Purkinje cells

Koike, Masato[1]; Uchiyama, Yasuo[1,2] ([1]*Juntendo Univ. Grad Sch. Med., Tokyo, Japan;* [2]*Juntendo Univ. Grad Sch. Med., Tokyo, Japan*)

We previously generated conditional cathepsin D (CD) or Atg7-deficient mice specifically in Purkinje cells (PCs). In both lines of mice, PCs underwent degeneration. Interestingly, CD-deficient PCs largely disappeared until 2 months of age, whereas Atg7-deficient PCs still survived at that time. Immunohistochemical observations exhibited that axonal spheroids and swellings in presynaptic terminals were more pronounced in Atg7-deficient PCs than in CD-deficient PCs. Electron microscopy also demonstrated that abnormal tubular vacuoles, nascent autophagosome-like structures, and membrane-bound and electron-dense granules accumulated within axons and presynaptic terminals of PCs in both lines of mice. Immnohistochmical analyses revealed that such spheroids and/or swellings in axon terminals of CD-deficient PCs are positive for both LC3 and Atg9A, a membrane protein essential for autophagy, while those of Atg7-deficient PCs are only positive for Atg9A. Moreover, immunoelectron microscopy using ultrathin cryosections confirmed that such tubular vacuoles are positive for Atg9A and inositol triphosphate receptor (IP3R), indicating that these abnormal tubular vacuoles are derived from the smooth endoplasmic reticulum (sER). From these results we propose the possibility that the origin of the autophagosomal isolation membrane in axons is derived from sER.

(COI: No)

S64-4

Microglial environment and fate of injured neurons

Konishi, Hiroyuki[1,2]; Kobayashi, Masaaki[1]; Kiyama, Hiroshi[1,2] ([1]*Nagoya Univ. Grad. Sch. of Med, Nagoya, Japan;* [2]*CREST, JST, Japan*)

Microglia continuously survey the microenvironment using their motile processes. They quickly respond to neuronal injuries and become activated. Activated microglia are assumed to have neurotoxic or neuroprotective effects; however, the signal determining how activated microglia affect the fate of neuronal cells remains largely unknown. We recently identified several molecules as crucial regulators of microglial neurotoxicity after neuronal injuries. In this symposium, we will present a transmembrane protein DNAX-activating protein of 12 kDa (DAP12) among those molecules. DAP12 functions as an adaptor protein by forming complexes with specific membrane receptor proteins such as triggering receptors expressed on myeloid cells 2 (TREM2), and transduces signals into the cytoplasm. Using a mouse hypoglossal axotomy model, we revealed that DAP12 was specifically expressed in activated microglia by the injury. The duration of microglial activation after nerve injury was decreased in DAP12-deficient mice, although cell morphology and total cell numbers were not affected at the activation peak. Furthermore, expression of M1-phenotype markers including pro-inflammatory cytokines was suppressed in DAP12-deficient microglia both in vitro and in vivo. Consequently, axotomy-induced motor neuron death was markedly prevented in DAP12-deficient mice. These results suggest that DAP12-mediated microglial activation following axotomy promotes pro-inflammatory responses, and thereby exacerbates neurotoxicity. Collectively microglia could be a potent regulator, which determines a fate of injured motor neurons.

(COI: No)

S64-5

A big channel in small glia as a promising molecular target for the treatment of opioid-induced hyperalgesia

Hayashi, Yoshinori; Nakanishi, Hiroshi (*Dept of Aging Sci and Pharmacol, Facl of Dent Sci, Kyushu Univ, Fukuoka, Japan*)

BK channels are the intracellular Ca^{2+} and voltage gated potassium channel. They are widely distributed throughout the nervous system to control neuronal excitability and neurotransmitter release. They are also expressed in electrically non-excitable cells such as cancer cells and immune cells, whereas little is known about their functions. Recently, we have found that large outward currents mediated by BK channels in the spinal microglia contribute to the initiation of neuropathic pain. In the present study, we have examined a possible involvement of microglial BK channels in opioid-induced hyperalgesia, because some evidence suggests the involvement of microglia in this event. Repeated morphine administration gradually enhanced pain sensitivity. At the same time, repeated morphine administration activated BK channels in microglia, but not in neuron, by generation of arachidonic acid and its metabolites through μ receptors. Morphine-induced hyperalgesia was significantly suppressed by BK channel inhibitor. The development of hyperalgesia was accelerated by intrathecal administration of morphine-primed wild-type, but not BK channel-deficient, microglia. Furthermore, the activation of BK channels promoted $P2X_4$ receptor trafficking to the cell surface of microglia. These results indicate that BK channels in the spinal microglia also play an important role in the development of opioid-induced hyperalgesia. Therefore, the BK channel is a potential molecular target for the treatment of both neuropathic pain and opioid-induced hyperalgesia.

(COI: No)

Symposium 65

Recent insight into molecules involved in food intake, stress and emotion

(March 23, 15:00～16:30, Room I)

S65-1

Alteration in RNA editing of serotonin 2C receptors is involved in alcohol drinking in mice

Tanaka, Masaki; Watanabe, Yoshihisa (*Kyoto Pref. Univ. Med., Kyoto, Japan*)

Serotonin 2C receptor (5-HT2CR) is a G-protein coupled receptor known to have various actions such as involvements in food intake, emotional behavior and drug addiction. We have recently demonstrated that 5-HT2CR is involved in the increased alcohol intake after chronic alcohol exposure in C57BL/6J strain. 5-HT2CR is also known to undergo mRNA editing that converts genomically encoded adenosine residues to inosines by adenosine deaminases acting on RNA (ADARs). We will present our data in the conference that alcohol preference in mice depends on the degree of 5-HT2CR mRNA editing in the nucleus accumbens (ACC), a crucial region for reward and addiction. We have recently demonstrated that 5-HT2CR in the ACC is involved in the increased alcohol intake after chronic alcohol exposure in C57BL/6J strain. After chronic alcohol vapor exposure for 20 days, C57BL/6J mice grew to take more alcohol voluntarily but C3H/HeJ and DBA/2J mice did not show significant changes. The frequency of 5-HT2CR RNA editing in the ACC of alcohol exposed mice was significantly increased in the C57BL/6J strain accompanied by the increase in the expression of 5-HT2CR mRNA, ADAR1 and ADAR2 but that was not observed in the C3H/HeJ nor DBA/2J strains. Then, we examined the mutant mice that express exclusively unedited type (INI) of 5-HT2CR mRNA in C57BL/6J strain and found that they did not exhibit the increase of alcohol intake compared with wild type after chronic alcohol exposure. Collectively, these results indicate that the alteration in 5-HT2CR mRNA editing in the ACC underlies the alcohol preference in mice.

(COI: No)

S65-2

Roles of the medial amygdala in the control of neuroendocrine responses to conditioned fear stimuli

Onaka, Tatsushi; Yoshida, Masahide; Takayanagi, Yuki (*Div Brain and Neurophysiol, Dept Physiol, Jichi Med Univ, Tochigi, Japan*)

Fear responses play important roles in maintaining homeostasis in response to threatening events. Conditioned fear stimuli induce freezing behaviour and neuroendocrine responses such as release of ACTH and oxytocin. During fear conditioning, information of a conditioned stimulus and an aversive stimulus converges in the basolateral amygdala. As a result, an initially neutral conditioned stimulus acquires aversive properties. The basolateral amygdala sends signals to the central amygdala, from which projections control expression of freezing behaviour. Selective lesions of the central amygdala have been shown to block expression of freezing behaviour while the lesions after conditioning have been reported not to impair release of corticosterone or prolactin. Thus, output nucleus of the amygdala remain to be determined concerning the control of neuroendocrine fear responses. Here, we examined excitotoxic lesions of the medial amygdala in the control of neuroendocrine responses to conditioned fear stimuli. We found that lesions of the medial amygdala impaired activation of medullary prolactin-releasing peptide (PrRP)-synthesizing neurons and blocked neuroendocrine conditioned fear responses. PrRP-deficient mice showed impaired neuroendocrine responses to conditioned fear stimuli. All these data suggest that medial amygdala-PrRP neuron pathway mediates, at least, in part neuroendocrine responses to conditioned fear stimuli.

(COI: No)

S65-3

Central circuit mechanism that drives stress-induced autonomic responses

Nakamura, Kazuhiro[1,2]; Kataoka, Naoya[1] ([1]*Career-path Promotion Unit for young Life Scientists, Kyoto Univ, Kyoto, Japan;* [2]*PRESTO, JST, Japan*)

Psychological stress induces increases in body temperature, heart rate and blood pressure. Although these stress-induced autonomic responses are commonly observed in many mammals, their central circuit mechanisms have been unknown. Recently, we have identified a hypothalamomedullary neural pathway that mediates stress signaling to drive sympathetic thermogenesis in brown adipose tissue (BAT), hyperthermia and tachycardia. This pathway involves direct glutamatergic transmission from the dorsomedial hypothalamus (DMH) to sympathetic premotor neurons in the rostral medullary raphe (rMR). Blockade of this neurotransmission with drug nanoinjections diminished BAT thermogenic, hyperthermic and tachycardic responses to social defeat stress, a sociopsychological stress model, in rats. Optogenetic stimulation of the neurotransmission from the DMH to the rMR elicited increases in BAT thermogenesis, heart rate and blood pressure, mimicking stress responses. Interestingly, our histochemical analysis revealed stress-induced activation of two populations of DMH neurons: ones projecting to the rMR and the others projecting to the paraventricular hypothalamic nucleus (PVH), a neuroendocrine center. These results indicate that the DMH functions as a hub for stress signaling, with monosynaptic projections to the rMR for the sympathetic stress responses and to the PVH for stress hormone release.

(COI: No)

S65-4

Structure-function insight on the melanin-concentrating hormone receptor 1

Saito, Yumiko (*Gras.Sch.Int.Arts & Sci, Higashi-Hiroshima, Japan*)

Melanin-concentrating hormone (MCH) is a cyclic neuropeptide that was originally discovered as a hypothalamic hormone which causes paling of teleost fish skin. It was later found that mammalian MCH is abundantly present in lateral hypothalamus as known to be the center of feeding behavior. MCH binds to and activates two G protein-coupled receptors (GPCR), MCHR1 and MCHR2 in goldfish, flounder, zebrafish, Xenopus tropicalis, and human. In rodents, MCHR1 is the sole receptor expressed and promiscuously couples to Gaq and Gai/o proteins. Activation of these various downstream pathways may contribute to the diverse array of physiological processes regulated by MCH. The recent progress using genetic and pharmacological approaches has confirmed that the MCH-MCHR1 system is involved in energy homeostasis and possibly emotional processing, and is an exiting new target for the treatment of obesity and certain psychological disorders. Therefore, the complex molecular and structural changes that provide receptor activation and signaling are the focus of intense research. In this symposium, I review the current knowledge regarding: i) the common and distinguishing structural features of MCHR1 for receptor activation and G protein selectivity and ii) the fine-tuning mechanism of MCHR1 activity by receptor-interacting molecules and the physiological significance.

(COI: No)

S65-5

Novel food-derived bioactive peptides acting on the nervous system

Ohinata, Kousaku (*Grad.Sch.Agri.Kyoto Univ., Kyoto, Japan*)

It is known that endogenous bioactive peptides are produced from precursor proteins after cleavage by specific proteases. Food proteins were not recognized as such precursor proteins; however, so far, a number of bioactive peptides released from food proteins have been found. These peptides sometimes act on the nervous system to modulate emotional behaviour or food intake even after oral administration.

We previously reported that dipeptide YL (0.3 mg/kg, p.o.) exhibited potent anxiolytic-like activity, comparable to diazepam. Recently, it was revealed that YL exhibits antidepressant-like activity, and increases neurogenesis in the hippocampus. Based on structure-activity relationship of YL-related peptides, we found several food-derived peptides with anxiolytic- and antidepressant-like activities. These YL-related peptides may activate serotonin 5-HT_{1A}, dopamine D_1 and $GABA_A$ system.

It is known that a decline in food intake is observed with aging, which is termed "anorexia of aging". In aged mice, ghrelin resistance was observed; however, rubiscolin-6 (1.0 mg/kg), a δ opioid agonist hexapeptide YPLDLF derived from a major green leaf protein Rubisco, stimulated food intake oral administration. Rubiscolin-6 may stimulate food intake activating the prostaglandin D_2-NPY pathway, independently of the ghrelin system.

During studies on mechanisms of food-derived peptides, novel pathways of the nervous system were also identified, suggesting that these peptides are unique research tools for studying neural pathways.

(COI: No)

S66-2

Angiotensin converting enzyme 2 (ACE2) links Apelin and angiotensin systems in controlling heart function

Kuba, Keiji (*Dept Biochem Metabolic Sci, Akita Univ Grad Sch Med, Akita, Japan*)

Angiotensin converting enzyme 2 (ACE2) is a negative regulator of the renin-angiotensin system (RAS), to catalyze conversion of Angiotensin II to Angiotensin 1-7. Apelin is a second catalytic substrate for ACE2 and functions as an inotropic and cardioprotective peptide. While an antagonistic effect between the RAS and the Apelin systems has been proposed, the functional interplay between Apelin and ACE2 remains elusive. Here we show that ACE2 is significantly down-regulated in Apelin deficient mice. Metabolomic profiling of angiotensin peptides showed similar down-regulation of Angiotensin 1-7 in Apelin mutant and ACE2 knockout mice. Pharmacological or genetic inhibition of Angiotensin II type 1 receptor (AT1R) rescues the impaired contractility and hypertrophy of Apelin mutant mice, accompanied with restored ACE2 levels. Importantly, treatment with Angiotensin 1-7 rescues hypertrophy and heart dysfunction in Apelin knockout mice. Moreover, Apelin treatment up-regulates ACE2 expression in failing hearts, and Apelin, via activation of its receptor APJ, increases ACE2 promoter activity in vitro. Apelin treatment also increases cardiac contractility and ACE2 levels in AT1R knockout mice. These data demonstrate that ACE2 couples the RAS to the Apelin system, adding a novel conceptual framework of the Apelin-ACE2-Angiotensin 1-7 axis as therapeutics for cardiovascular diseases.

(COI: No)

Symposium 66

Update of Research on Cardiovascular Regulation by Angiotensin

(March 23, 16:30～18:00, Room A)

S66-1

Cross Talk between the Hypoxia Response System and Angiotensin II Receptor

Ichiki, Toshihiro (*Dep Cardiol, Harasanshin HP, Fukuoka, Japan*)

Background: Prolyl hydroxylase domain-containing protein (PHD) induces proteasomal degradation of hypoxia-inducible factor (HIF)-α, a transcription factor. Inhibition of PHD stabilizes HIF-α expression and increases the expression of target genes such as vascular endothelial growth factor. We examined the effect of PHD inhibition on the expression of angiotensin (Ang) II type 1 receptor (AT1R) and cardiovascular remodeling.

Results: Hypoxia (1% O_2), cobalt chloride ($CoCl_2$), a hypoxia mimetics that inhibits PHD, and knockdown of PHD2, a major isoform of PHDs, by siRNA reduced AT1R expression in vascular smooth muscle cells. Oral administration of $CoCl_2$ to mice treated with Ang II significantly reduced aortic AT1R expression and perivascular fibrosis of coronary artery.Mice with myeloid-specific deletion of PHD2 (MyPHD2KO) were generated, resulting in the accumulation of HIF-1α and 2α in macrophages. Cardiac interstitial fibrosis, macrophage infiltration and myocyte hypertrophy induced by L-NAME, a nitric oxide synthase inhibitor, and Ang II treatment were significantly ameliorated in MyPHD2KO mice. Left ventricular hypertrophy and dysfunction induced by L-NAME/Ang II treatment in control mice were not observed in MyPHD2KO mice.

Conclusions: PHD inhibition downregulates AT1R expression and attenuates profibrotic effect of Ang II. Phd2 deletion in myeloid lineage attenuates cardiac hypertrophy and fibrosis, which may be mediated by decreased inflammation- and fibrosis-associated gene expression in macrophage. PHD inhibition may be a novel strategy for ameliorating cardiovascular remodeling through AT1R suppression.

(COI: No)

S66-3

Regulation of L-type Ca^{2+} channels by angiotensin II type 1 receptor/β-arrestin-2 biased signaling through casein kinase 2 in immature cardiomyocytes

Kashihara, Toshihide; Nakada, Tsutomu; Guo, Xiaoguang; Yamada, Mitsuhiko (*Dept Mol Pharmacol, Shinshu Univ Sch Med, Matsumoto, Japan*)

Angiotensin II (AII) plays important roles in cardiovascular functions. In this study, we examined the effect of AII on L-type Ca^{2+} channels (LTCC), which play a pivotal role in cardiac excitation-contraction coupling. 2-hour treatment of AII (3μM) doubled LTCC activity in mouse neonatal ventricular myocytes (NVMC) and immotile mouse atrial cell line HL-1, but not in isolated adult ventricular myocytes (AVMC). An AT_1 receptor blocker, candesartan (10μM), but not an AT_2 receptor blocker, PD123319 (3μM), abolished the effect of AII in NVMC and HL-1. In HL-1, knockdown of β-arrestin-2 but not β-arrestin-1, G_q or G_{11} significantly inhibited the effect of AII. PKC inhibitor, Go6983 (0.5μM), also did not affect the effect of AII. It is reported that in cardiomyocytes, AII promotes the proteosomal breakdown of cyclin-dependent kinase inhibitor 1B (p27), thereby activating casein kinase 2α' (CK2α') associated with this protein. Indeed, knockdown of p27 caused AII-independent activation of LTCC whereas knockdown of CK2α' resulted in significant inhibition of the effect of AII on LTCC in HL-1. The expression of CK2α' was 7 and 4.5 times higher in NVMC and HL-1, than in AVMC, respectively. Furthermore, the inhibition of Src family tyrosine kinases, which promote p27 degradation, with bostinib (2μM) significantly inhibited the effect of AII on LTCC in HL-1. These results indicate that AT_1 receptor/β-arrestin-2/Src/p27/CK2α' strongly activates LTCC in immature but not adult cardiomyocytes.

(COI: No)

S66-4

Angiotensin II receptor blocker (ARB)-sensitive and insensitive remodeling in hearts of inherited DCM mice

Kurebayashi, Nagomi[1]; Odagiri, Fuminori[1,2]; Inoue, Hana[3]; Sugihara, Masami[1,2]; Suzuki, Takeshi[1,2]; Murayama, Takashi[1]; Shioya, Takao[4]; Konishi, Masato[3]; Morimoto, Sachio[5] (*[1]Dept Pharmacol, Juntendo Univ Sch Med, Tokyo, Japan; [2]Dept Cardiol, Juntendo Univ; [3]Dept Physiol, Tokyo Med Univ; [4]Dept Physiol, Fac Med, Saga Univ; [5]Dept Clin Pharmacol, Fac Med Sci, Kyushu Univ*)

Inherited dilated cardiomyopathy (DCM) is a progressive disease often results in sudden death (SD) or heart failure (HF). Although angiotensin receptor blockers (ARBs) have been used for the treatment of HF, the effects of ARB on postulated electrical remodeling in inherited DCM are not well known. We examined effects of candesartan (CAND), one of the ARBs, on structural and electrical remodeling in hearts of inherited DCM mice (TNNT2 ΔK210). DCM mice were treated with CAND from 1 month of age. Non-treated DCM mice showed cardiac enlargement with prolongation of QRS and QT intervals, and died at $t_{1/2}$ of 70 days. CAND greatly suppressed cardiac dilatation, prolongation of QRS and QT interval and SD with lethal arrhythmia, and dramatically extended lifespan of DCM mice. Expression analysis revealed that down-regulation of Kv4.2 (I_{to}), and Kv1.5 (IK_{ur}) in DCM was partially reversed by CAND. Interestingly, non-treated DCM heart had both normal-sized myocytes with moderately reduced I_{to} and IK_{ur} and enlarged cells with greatly reduced K^+ currents (I_{to}, IK_{ur} IK_1 and IK_{SS}). CAND treatment completely abrogated the emergence of the enlarged cells but did not reverse the I_{to}, and IK_{ur} in normal-sized cells. ARB-sensitive and insensitive remodeling were found in DCM hearts. CAND treatment suppresses severe electrical remodeling related to structural remodeling in inherited DCM.

(COI: No)

Symposium 67

Central functions of oxytocin: Basic and clinical neuroscience

(March 23, 16:30～18:00, Room C)

S67-1

Oxytocin as a therapeutic target for depression and other mental disorders

Matsui, Hideki; Matsushita, Hiroaki (*Dept Physiol, Grad Sch Med, Okayama Univ Med Sch, Okayama, Japan*)

Oxytocin (OT) acts as a neurotransmitter/neuromodulator to regulate a diverse range of central nervous system (CNS) functions, including emotional and social behavior. Clinical reports suggest OT to be a promising drug for psychiatric diseases such as depression, anxiety disorders and autism. A recent study found that sexual activity with a female induced the release of OT in the CNS of male rats. Moreover, a drug for the treatment of human with sexual dysfunction, sildenafil, induces enhancement of OT release from the CNS of mammals. Sildenafil is a selective inhibitor of PDE5 enzyme. In this study, we examined the effect of mating behavior on depression-related behavior in wild-type (WT) and OT receptor-deficient (OTR KO) male mice. The WT mice showed a reduction in depression-related behavior after mating behavior, but the OTR KO mice did not. Moreover, application of sildenafil reduced depression-related behavior in male mice. The antidepressant-like effect was absent in OTR KO mice. The activation of a MAP kinase cascade and subsequent enhanced phosphorylation of CREB in the hippocampus have been proposed as common mediators of antidepressant efficacy. Sildenafil increased the phosphorylation of CREB in the hippocampus. These results suggest mating behavior and sildenafil have an antidepressant effect through activation of OT signaling pathway.

(COI: No)

S67-2

Oxytocin projections regulate the spinal gastrin-releasing peptide system that controls male sexual function

Sakamoto, Hirotaka (*Grad.Sch.Nat.Sci. & Tech.Okayama Univ., Okayama, Japan*)

We previously demonstrated that the gastrin-releasing peptide (GRP) system in the spinal cord influences spinal centers promoting penile reflexes in rats. The paraventricular nucleus (PVN) of the hypothalamus contains the somata of oxytocin (OT) neurons that project to the posterior pituitary, from which OT is released into blood vessels. Additionally, a group of OT neurons in the PVN also projects to the spinal cord. Therefore, the hypothesis has been proposed that OT, which is transported by long descending paraventriculospinal pathways, activates proerectile spinal centers. However, the direct linkage of the neural circuit between the hypothalamic PVN and penile innervation remains uncharacterized. Hence, the purpose of this study is to reveal the function of OT in the brain-spinal cord neural network controlling male sexual function. First, we found that the axonal distribution of OT in the lumbar spinal cord exhibits a male-dominant sexual dimorphism in rats. Furthermore, OT binding and expression of the specific OT receptor were observed in the somata of spinal GRP neurons. Consequently, we studied the expression of phosphorylated ERK (pERK) in the GRP neurons after ejaculation. This revealed that pERK induction in the GRP neurons appeared to be specifically associated with ejaculation, suggesting that secreted oxytocin in the lumbar spinal cord activates the GRP neurons during male sexual behavior. Taken together, these results suggest that the hypothalamic OT projections mediate the GRP system in the lumbar spinal cord that controls male sexual function.

(COI: No)

S67-3

Roles of oxytocin in the control of emotional and social behaviors

Takayanagi, Yuki; Yoshida, Masahide; Onaka, Tatsushi (*Div Brain and Neurophysiol, Dept Physiol, Jichi Med Univ, Tochigi, Japan*)

Various stressful stimuli, including conditioned fear stimuli, electric foot shocks, or restraint stress, have been shown to activate oxytocin neurons and facilitate oxytocin release. We have demonstrated that noradrenergic neurons in the medulla oblongata enhance oxytocin release in response to some stressful stimuli. An administration of oxytocin reduces anxiety-related behavior and attenuates the HPA axis. These data suggest that oxytocin released in response to stressful stimuli might exert anti-stress actions. On the other hand, social stimuli also have been shown to activate oxytocin neurons although the neural pathways for activation of oxytocin neurons in response to social stimuli are largely unknown. Oxytocin release is facilitated by administration of secretin, which is implicated in the control of social behavior. We investigated whether secretin regulates social behavior via the oxytocin system. An intracerebroventricular injection of secretin activated supraoptic oxytocin neurons. Application of secretin facilitated oxytocin release dendritically in an *in vitro* preparation of the supraoptic nuclei. Furthermore, local application of secretin into the supraoptic nucleus facilitated social recognition and its action was blocked by an oxytocin receptor antagonist injected into the medial amygdala. These results suggest that secretin activates supraoptic oxytocin neurons, potentiates dendritic oxytocin release, and facilitates social recognition via the oxytocin/oxytocin receptor system.

(COI: No)

S67-4

Clinical study to develop oxytocin as a candidate for therapeutics of core symptoms in autism spectrum disorders

Yamasue, Hidenori (*Dept. Neuropsychiatry, Grad. Sch. of Med., Tokyo Univ., Tokyo, Japan*)

Autism spectrum disorders, which prevail as high as 1 in 100 individuals, currently have no established pharmacological treatment. Previous preliminary studies have suggested therapeutic effects of oxytocin on the disorders. The speaker's research team further explored neural evidence for the therapeutic effects of the neuropeptide by examining oxytocin's effects on socio-communicational deficits, which constitute the core symptoms of autism spectrum disorders. In our double-blind, placebo-controlled, crossover trial involving 40 high-functioning adults with autism spectrum disorders, intranasal administration of oxytocin behaviorally mitigates autistic deficits in understanding social communication contents, such as irony and humor, whose verbal and nonverbal information is conflicting, by recovering originally-diminished brain activities, enhancing functional connectivity and affecting neurochemical aspects of neuronal markers in the area. Based on the findings from the trial of single dose administration, we further conducted a trial of long-term treatment of intranasal oxytocin to further establish clinical application of it as a therapeutic for core symptoms of autism spectrum disorders. In the symposium, further discussion on such possibility would be expected.

(COI: No)

S67-5

Oxytocin signal molecules: Physiology and pathophysiology

Higashida, Haruhiro (*Research Center for Child Mental Development, Kanazawa University, Kanazawa, Japan*)

I demonstrated that CD38, a transmembrane protein with ADP-ribosyl cyclase activity, plays a critical role in mouse social behavior by regulating the release of oxytocin (OXT), which is essential for mutual recognition. When CD38 was disrupted, social amnesia (Jin et al., Nature, 2007) and less paternal nurturing behavior (Akther et al., Mol. Brain, 2014) were observed in CD38 knockout mice. CD38 knockout sires failed to retrieve their pups when they were reunited after cohabituated separation in a new cage for 10 min. CD38 knockout sires treated with a single subcutaneous injection of OXT partially rescued the retrieval events when co-housed with CD38 knockout sires treated with OXT. Next, we examined the effect of local expression of human CD38 in the nucleus accumbens (NAcc) in males via lentiviral infection. Pairs of knockout dams treated with OXT and sires expressing CD38 in the NAcc displayed more retrieval. A complete recovery was obtained both by sires with the expression of CD38 in the NAcc and with OXT administration. After identifying the families of the retrievers or non-retrievers, c-Fos expression in neuronal subsets in the mPOA, ventral tegmental area, NAcc and ventral palladium was much higher in the retriever sires when they isolated together with their mates in new cages. Finally, I discuss that single nucleotide polymorphysims in CD38 may be possible risk factors for autism spectrum disorder by abrogating OXT function and that some ASD subjects can be treated with OXT in preliminary clinical trials.

(COI: No)

Symposium 68

Diversity of serotonergic system in the brain - from development to aggression, reward and decision-making -

(March 23, 16:30～18:00, Room E)

S68-1

Serotonin as a trophic factor for the development of the behavior

Shiga, Takashi (*Fac. Med., Univ. Tsukuba, Tsukuba, Japan*)

Serotonin (5-hydroxytryptamine, 5-HT) is a neuromodulator in the adult brain. 5-HT and its receptors appear early in the developing brain, and 5-HT acts as a neurotrophic factor. In addition, the abnormality of 5-HT system leads to psychiatric disorders, such as anxiety disorder and depression, and learning disability. We have reported that the treatment of fluoxetine, a selective 5-HT reuptake inhibitor, during the early postnatal period decreased both the anxiety-like behavior in the elevated plus maze and the depression-like behavior in the forced swim test in adult BALB/c mice. The same treatment also improved spatial learning in Morris water maze. These results suggest that 5-HT system during the development affect various behaviors after maturation. Interestingly, the fluoxetine treatment of C57 BL/6 mice did not change the stress response and the learning ability. These strain differences may be due to the higher amount of the brain 5-HT in C57BL/6 mice as compared with BALB/c mice. Among the 5-HT receptors, 5-HT1A receptor may be responsible for the regulation of anxiety, because the treatment of the BALB/c mice with 5-HT1A receptor agonist decreased the anxiety-like behavior. The role of 5-HT1A receptor is also supported by the experiments using the 5-HT1A receptor-KO mice. Because 5-HT1A receptor agonist treatment increased the depression-like behavior, this receptor may regulate negatively the depression. These results suggest that different 5-HT receptors may be involved in the anxiety and depression.

(COI: No)

S68-2

Serotonin and aggressive behavior: from laboratory animal to human

Ueda, Shuichi; Kai, Nobuyuki; Yamaguchi, Tsuyoshi; Ehara, Ayuka; Tachibana, Atsumichi (*Dokkyo.Med.Univ., Tochigi, Japan*)

Aggressive behavior is an instinctive and essential behavior in many mammalian species, and can be classified into two categories: predatory aggression and affective aggression by Moyer (1968). Predatory aggression is similar to human premeditated violence, which represents a planned behavior with low autonomic response, whereas affective aggression is similar to impulsive aggression, which is reactive and associated with high autonomic response. Serotonin (5-HT) is an important neurotransmitter and/or neuromodulator associated with a wide range of behaviors. In particular, a negative correlation between brain 5-HT activity and aggressive behavior has been studied. We previously reported that fetal 5-HT neurons transplanted into the rat hypothalamus restored inhibition of predatory aggression that have been induced by lesion with 5, 7-dihydroxytryptamine into dorsal and medial raphe nuclei. Reinnervation of 5-HT fibers in the lateral hypothalamus (LH) from the grafted neurons resulted simultaneously with a significant reduction of c-Fos expression in the LH neurons. These results indicate the possibility that 5-HT neurons regulate predatory aggression through the inhibition of the activity of the LH neurons. On the other hand, affective aggression is seemed to regulate by two monoamines, 5-HT and dopamine (DA), with opposing roles. Hyperactivity of DA system is associated with increased affective aggression, while 5-HT system inhibits affective aggression.

(COI: No)

S68-3

Neuronal activity of dorsal raphe nucleus during reward schedule

Shidara, Munetaka[1,2] ([1]*Systems Neurosci, Facul Med, Univ Tsukuba, Tsukuba, Japan;* [2]*Kansei Behav Brain Sci, Grad Sch Compreh Human Sci, Univ Tsukuba, Tsukuba, Japan*)

Dorsal raphe nucleus is a major source of serotonin neurons which are related to emotion, appetite, stress, aggressive behavior, mental disorder, and so on. It is only recently that physiologists have begun to investigate the dorsal raphe's possible role in reward processing. Here, we examined whether dorsal raphe neurons showed differential activities between the conditions when the monkey could or could not predict reward availability and amount. We recorded from 98 single neurons in dorsal raphe of two monkeys during a multi-trial reward schedule task. In the task, the monkeys were required to perform a visual discrimination trial 1, 2 or 3 times (schedule) for obtaining liquid reward of 1, 2 or 3 drops. In the cued condition, the length and brightness of the cue indicated schedule progress and reward amount, respectively. In the random condition, the cue was randomly presented with respect to schedule length and reward amount, so that the monkeys could not predict the reward schedule and amount. We found the neurons encoding the information about schedule onset, reward expectation, reward outcome, and reward amount in the mean firing rates. Furthermore, information theoretic analysis showed that the temporal pattern of neuronal responses contained additional information about the schedule progress. These results suggest that the dorsal raphe neurons have important roles on reward information processing and, considering the diverse anatomical connection, possibly providing signals throughout the brain to coordinate persistent goal-seeking behavior.

(COI: No)

S68-4

Serotonin and patience

Miyazaki, Katsuhiko; Miyazaki, Kayoko W; Doya, Kenji (*Neural Computation Unit, OIST, Okinawa, Japan*)

Recent recording and pharmacological inhibition studies of serotonin neurons in the dorsal raphe nucleus (DRN) have shown that these neurons play roles in promoting actions for future rewards. Here we developed mice that express channelrhodopsin-2 in the serotonin neurons and showed that the selective activation of the serotonin neurons in the DRN enhanced the mice's patience in waiting for both the conditioned reinforcer tone at a tone site and the food reward at a food site. Optogenetic activation of DRN serotonin neurons while the mice waited for the tone by keep nosepoking at the tone site significantly reduced the number of tone wait errors. When serotonin neurons were activated during the variable delay periods when the mice waited for the food by keep nosepoking at the food site (3, 6, or 9 sec or infinity, i.e., omission), the reward wait errors were significantly reduced in the 9 sec waiting trials. In the reward omission trials, the waiting time of the mice was significantly longer (17.5 sec; mean) in the serotonin activation trials compared with the trials with no activation (12.0 sec). This effect was observed specifically when the animal was engaged in deciding whether to keep waiting and not due to motor inhibition. Control experiments showed that the prolonged waiting times observed with optogenetic stimulation were not due to behavioral inhibition or the reinforcing effects of serotonergic activation (Miyazaki et al., Curr Biol, 2014). These results indicate that the temporally precise activation of the serotonin neurons during waiting facilitates patience for delayed rewards.

(COI: No)

S68-5

Appetitive and aversive information coding in the primate dorsal raphé nucleus

Nakamura, Kae; Hayashi, Kazuko; Nakao, Kazuko; Noritake, Atsushi (*Dept. Physiol. Kansai Medial University Osaka Japan*)

There have been conflicting hypotheses about whether the central serotonergic system is involved in appetitive or aversive information processing. To reveal whether and how such opposing information processing can be achieved by single neurons in the dorsal raphé nucleus (DRN), the major source of serotonin in the forebrain, we measured the activity of these neurons while monkeys were conditioned in a Pavlovian procedure with two distinct contexts: an appetitive context where a reward was available and an aversive one where an airpuff was delivered. We found that single DRN neurons were involved in distinct aspects of appetitive and aversive information processing. First, more than half of the recorded DRN neurons discriminated appetitive and aversive contexts by tonic changes in activity. In the appetitive context, they then kept track of expected reward value indicated by the conditioned stimuli. Some of them also encoded an error between the obtained and expected values. In the aversive context, the same neurons maintained tonic modulation in their activity throughout the block. However, modulation of their responses to trial events depending on airpuff probability was not common. Taken together, these results indicate that single DRN neurons encode both appetitive and aversive information, but over differing time scales, relatively shorter for appetitive and longer for aversive. Such dynamic ranges of information processing performed by single DRN neurons may contribute to the integral role of the serotonergic system in decision making in different emotional contexts.

(COI: No)

Symposium 69

New trends for research on the regulatory mechanism of neuronal development

(March 23, 16:30～18:00, Room G)

S69-1

Roles of volume-regulated anion channels during neuronal migration in the developing brain

Akita, Tenpei; Furukawa, Tomonori; Fukuda, Atsuo (*Dept Neurophysiol, Hamamatsu Univ Sch Med, Hamamatsu, Japan*)

Neuronal migration is the process regulating coordinated changes in neuronal morphology. Such morphological changes inevitably include the changes in neuronal cell volume both locally and globally, but how the cell volume during neuronal migration is regulated is not elucidated yet. Cell volume regulation is attained by regulating the net influx and efflux of solutes and water across the plasma membrane. As a pathway for anion flux during the volume regulation, the volume-sensitive outwardly rectifying (VSOR) anion channel is known to play a major role in almost all types of vertebrate cell. But its role in the developing brain remains to be investigated. We recently confirmed that VSOR anion channels indeed work in the neurons in the developing brain. The VSOR anion channel is permeable not only to Cl^- ions, but also to amino acids like glutamate, aspartate and taurine. Taurine is abundant in the developing brain and we recently found that taurine acts as a major endogenous agonist of $GABA_A$ receptors in the developing brain to control the speed of radial migration of the neurons, especially in the subplate region of the developing neocortex. We further confirmed that VSOR anion channels provide the pathway for taurine release to regulate the radial migration. Thus the VSOR anion channel plays dual roles; one is in cell volume regulation and another is in intercellular communication, to regulate neuronal migration in the developing brain.

(COI: No)

S69-2

Control of cerebellar granule cell migration by intrinsic programs through modulating Ca^{2+} and cyclic nucleotide signaling

Kumada, Tatsuro (*Dept Occup Ther, Tokoha Univ, Hamamatsu, Japan*)

In the developing brain, immature neurons migrate from their birthplace to their final destination. On the way to their destination, neurons changes the speed and direction of cell movement in a cell type and cortical layer-specific manner. External guidance cues allow migrating neurons to restrain and guide in local milieu, but little is known about the role of intrinsic signals in controlling neuronal migration. To elucidate this issue, we have investigated relationship between behavior of migrating neurons and second messenger signaling (specifically Ca^{2+} and cyclic nucleotide signaling) in cerebellar granule cell migration. Previously, we demonstrated that Ca^{2+} spike frequency can control the cortical layer specific alterations of granule cell migration and loss of Ca^{2+} spikes is required to complete neuronal migration. Interestingly, our results suggest that intrinsic programs can determine the timing of Ca^{2+} spike loss. Thus, we also examined the role of intrinsic signals in neuronal cell turning. Time-lapse imaging of individual granule cell movement in microexplant culture revealed that these cells periodically turned without cell-cell contact and in the absence of potential guidance cues. These observation revealed four distinct modes of cell turning. Remarkably, the occurrence of each mode of turning was differentially controlled by the orchestrated activity of multifarious signaling pathways through Ca^{2+} and cyclic nucleotide signaling. These results suggest that intrinsic programs provide prerequisite signals for neuronal migration.

(COI: No)

S69-3

Transcription factor Npas4 regulates the sensory experience-dependent development of dendritic spines in newborn olfactory bulb interneurons

Yoshihara, Sei-ichi[1]; Takahashi, Hiroo[1]; Nishimura, Nobushiro[1]; Kinoshita, Masahito[1]; Asahina, Ryo[1]; Hibi, Yoko[2]; Nagai, Taku[2]; Yamada, Kiyofumi[2]; Tsuboi, Akio[1]
([1]*Lab for Mol Biol of Neural System, Nara Med Univ, Kashihara, Japan;* [2]*Dep of Neuropsychopharm and Hosp Pharm, Nagoya Univ, Nagoya, Japan*)

Sensory experience regulates development in various brain structures, including the cortex and olfactory bulb (OB). However, little is known about the developmental role of sensory experience in the OB GABA-releasing inhibitory interneurons, such as granule cells (GCs). In this study, by *in situ* hybridization (ISH) screenings, we newly identified a transcription factor, *Npas4* gene, which is expressed in a subset of OB GCs following sensory experience. Then, we performed the gain- and loss-of-function experiments for *Npas4* in OB GCs, based on lentiviral injection. *Npas4* overexpression in newborn OB GCs increased the spine density even under sensory deprivation. Conversely, both *Npas4* knockdown and knockout resulted in a significant reduction in the spine density of OB GCs. In addition, by ChIP-seq plus ISH screenings, we identified, as a novel target of Npas4, an E3 ubiquitin ligase *Mdm2* gene, which is expressed at low levels in the wild-type OB but at higher levels in the *Npas4*-knockout OB. Proteomics analysis further revealed that Mdm2 ubiquitinates and degrades Dcx to reduce the dendritic spine density of OB GCs. Taken together, our findings suggest that *Npas4* regulates *Mdm2* expression to ubiquitinate and degrade Dcx for shaping the dendritic spines of OB GCs after sensory experience (Yoshihara *et al*, **Cell Reports**, 8, 843-857, 2014).

(COI: No)

S69-4

Roles of axon guidance molecule FLRT2 in development of vascular system

Yamagishi, Satoru[1]; Kubota, Yoshiaki[2]; Sato, Kohji[1] ([1]*Hamamatsu Univ. Sch. Med., Shizuoka, Japan;* [2]*Sch., Med., Keio Univ., Tokyo, Japan*)

Axon tracts and blood vessels course throughout the body in an elaborated and orderly pattern, often alongside one another. The mechanisms involved in wiring neuronal and vascular networks share the axon guidance molecules such as netrin/unc5, slit/robo, semaphorin/neuropilin, and ephrin/Eph. Recently we reported that the new axon guidance molecule FLRT2 repel upper layer neurons in order to suppress the radial migration during brain development. FLRT2 is cleaved at juxta-membrane region and bind to Unc5B/D receptors. Here we show that FLRT2 is involved in the formation of vascular system. FLRT2 mutants appear partial embryonic lethality with hemorrhage from mid- to late-gestation stage. Interestingly, at E9.5 the expression of adhesion molecule PECAM in endothelial cells in FLRT2 mutants is down-regulated. Furthermore, FLRT2 mutants also showed partial hypervascular phenotype during eye development. Finally, Unc5B-positive endothelial cells are repelled from FLRT2 in a stripe assay. These results suggest that FLRT2 plays important roles in vascular development not only as a repulsive guidance molecule but also as a regulator of the vascular integrity.

(COI: No)

S69-5

FLRT3 is a Robo1-Interacting Protein that Determines Netrin-1 Attraction in Developing Axons

Egea, Joaquim (*Univ. Lleida, IRBLLEIDA, Lleida, Spain*)

Guidance molecules are normally presented to cells in an overlapping fashion; however, little is known about how their signals are integrated to control the formation of neural circuits. In the thalamocortical system, the topographical sorting of distinct axonal subpopulations relies on the emergent cooperation between Slit1 and Netrin-1 guidance cues presented by intermediate cellular targets. However, the mechanism by which both cues interact to drive distinct axonal responses remains unknown.Here, we show that the attractive response to the guidance cue Netrin-1 is controlled by Slit/Robo1 signaling and by FLRT3, a novel coreceptor for Robo1. While thalamic axons lacking FLRT3 are insensitive to Netrin-1, thalamic axons containing FLRT3 can modulate their Netrin-1 responsiveness in a context-dependent manner. In the presence of Slit1, both Robo1 and FLRT3 receptors are required to induce Netrin-1 attraction by the upregulation of surface DCC through the activation of protein kinase A. Finally, the absence of FLRT3 produces defects in axon guidance in vivo.These results highlight a novel mechanism by which interactions between limited numbers of axon guidance cues can multiply the responses in developing axons, as required for proper axonal tract formation in the mammalian brain.

(COI: No)

Symposium 70

Activity-dependent regulation of myelinated nerve function and morphology

(March 23, 16:30～18:00, Room H)

S70-1

Molecular mechanisms of myelinated nerve formation and injury

Susuki, Keiichiro (*Boonshoft Sch. Med., Wright State Univ., Dayton, USA*)

The nervous system function in vertebrates depends on the interaction between neurons and glial cells forming myelin, a multi-lamellar structure surrounding axons. The action potentials are initiated at the axon initial segment, a highly specialized neuronal compartment in the proximal axon. The action potentials are then regenerated at the nodes of Ranvier, short gaps between two adjacent myelin segments, and propagate rapidly along the axon. Both axon initial segments and the nodes are characterized by highly accumulated molecular complex including voltage-gated ion channels. During development, the axon initial segments are intrinsically determined and assembled by the neurons through the restriction of the molecules by the distal axonal cytoskeleton. Nodes of Ranvier are formed by multiple mechanisms: interactions with extracellular matrix, paranodal diffusion barrier, and stabilization by the axonal cytoskeleton. Since axon initial segments and nodes of Ranvier are critical for the proper nervous system functions, the dysfunction and/or disruption of these domains lead to neurological symptoms. Recent evidences demonstrate that the altered expression or localization of molecules at axon initial segments and nodes are key contributors to the pathophysiology of various neurological and psychiatric disorders. Better understanding of mechanisms underlying myelinated nerve formation and injury will provide important clues to establish novel therapeutic approaches for currently intractable nervous system diseases.

(COI: No)

S70-2

Activity dependent myelination and impaired motor learning as the result of its disruption

Wake, Hiroaki (*NIPS, Okazaki, Japan*)

Myelin, a multilayered membrane insulation wrapped around the axons, increases axonal conduction velocity at least by 50 fold. Myelination around the axon is thought to be crucial for information processing by changing the timing of neural firing patterns during development and learning. Additionally, Stimulating myelination as a result of impulse activity in axons could enable myelin to be regulated by environmental experience, which could contribute to information processing in the brain. We have demonstrated that local translation of MBP mRNA in oligodendrocyte processes is initialized myelin formation at the site of connection between oligodendrocytes and axons depending on neural activity. These findings provide new insight into how myelination, and thus conduction velocity and function of neural circuits, can be regulated by nervous system activity. Then to consider how activity dependent myelination can be involved in information processing, we used myelin proteolipid protein 1 (PLP1) over expression mouse (PLP-tg). To understand the neural basis of the cognitive impairment caused by the reduction of the neural conduction velocity, we used two-month-old PLP-tg mice which have a slight reduction of conduction velocity and combined in vivo two photon microscopy with a motor learning task. GFP-based Calcium Calmodulin probe (G-CaMP) was induced by an adeno-associated virus (AAV) injection in layer 2/3 of the M1 cortex to enable detecting a difference in the firing pattern of neuronal activity with a lever pulling motor learning task.

(COI: No)

S70-3

Functional plasticity of white matter in the hippocampus

Yamazaki, Yoshihiko (*Dept Physiol, Yamagata Univ Sch Med, Yamagata, Japan*)

Structural plastic changes of white matter in the adult brain are received considerable attention in relation to normal cognitive function and learning. Oligodendrocytes and myelin can respond to neuronal activity with depolarization of membrane potential. We previously reported that repetitive depolarization of the oligodendrocyte increased the conduction velocity of axons it myelinated in rat hippocampus. These results indicate that white matter shows functional plasticity as well as structural plasticity and that the depolarization of oligodendrocytes is involved in the generation of functional plasticity. To investigate the functional plastic changes in the white matter, we used a mouse with channelrhodopsin-2 expression restricted to oligodendrocyte and examined the effects of oligodendrocyte depolarization on axonal conduction of action potentials. Using extracellular recordings of compound action potentials at the alveus of the hippocampus, we found that light-evoked depolarization of oligodendrocytes induced early- and late-onset facilitation of axonal conduction that was dependent on the magnitude of oligodendrocyte depolarization; the former lasted for approximately 10 min, whereas the latter continued for up to 3 h. Using whole-cell recordings from CA1 pyramidal cells and recordings of antidromic action potentials, we found that the early-onset short-lasting component included the decrease of conduction latency of action potentials. These modulatory effects of oligodendrocytes would promote synchrony among the axons, and may influence the information processing in the white matter.

(COI: No)

S70-4

Mitochondrial behavior in myelinated axons modulated by axonal electrical activity

Ohno, Nobuhiko[1,2] ([1]*Univ. Yamanashi, Yamanashi, Japan;* [2]*Nat. Inst. Physiol. Sci., Aichi, Japan*)

Myelination facilitates rapid propagation of axonal conduction by confining Na+-channel currents at nodes of Ranvier, and conserves ATP consumption needed to exchange axoplasmic Na+ for extracellular K+. To meet the energy demands of saltatory nerve conduction, myelination may alter transport and localization of axonal mitochondria, which is a major source of axonal ATP. The majority of axonal mitochondria are located at stationary foci which are distributed along the entire length of the axon, while a population of relatively small axonal mitochondria are translocated in both anterograde and retrograde directions. The transport and docking of axonal mitochondria are dynamic processes which can be modulated by axonal metabolic demands. In myelinated axons, stationary mitochondria are abundant in juxtaparanodal and internodal axoplasm where Na+/K+-ATPase as well as mitochondrial energy substrates are enriched. Upon increased axonal firing, motile mitochondria preferentially stopped near the nodes, and the stationary mitochondria can be increased in nodal axoplasm. The nodal stopping of motile mitochondria is mediated by increase of axoplasmic Ca2+, and perturbed when compact myelin formation is congenitally impaired. These results support the concept that myelination modulates mitochondrial behavior at the nodes to meet the metabolic demand of saltatory nerve conduction.

(COI: No)

S70-5

Spike initiation and conduction in auditory time-coding pathway

Kuba, Hiroshi[1,2] ([1]*Dept Cell Physiol, Grad Sch Med, Nagoya Univ, Nagoya, Japan;* [2]*JST PRESTO*)

Sound localization requires detecting a difference in sound arrival times between the two ears (ITD, interaural time difference). In birds, this calculation is made in a brainstem circuit that is composed of arrays of binaural coincidence detectors innervated with delay lines, which enables ITDs to be encoded as a place of active neurons within the circuit. Precise and reliable delivery of spikes to the coincidence detectors is critical for accurate ITD detection, and neurons in nucleus magnocellularis (NM) play the role. To accomplish their tasks, NM neurons are highly specialized in arrangements of the axon initial segment (AIS) and node of Ranvier, which are axonal compartments, accumulated with high density of voltage-gated Na channels and involved in initiation and conduction of action potentials, respectively. For example, length of the AIS is effectively coupled with synaptic inputs, which ensures precise and reliable initiation of spikes in individual neurons. On the other hand, inter-nodal length is known to differ along the axon in a region-specific manner; that is, the length is long outside the delay lines, while it becomes shorter at the delay lines, contributing to a fine regulation of conduction velocity. In this symposium, I will summarize our findings on these differentiations, and discuss how they are influenced by neuronal activity.

(COI: No)

Symposium 71

Regulation of appetite and energy metabolism by brain

(March 23, 16:30～18:00, Room I)

S71-1

Effect of GALP on lipid metabolism in the liver

Hirako, Satoshi[1]; Takenoya, Fumiko[2]; Kageyama, Haruaki[3]; Wada, Nobuhiro[1]; Shioda, Seiji[1] (*[1]Showa Univ. Sch. of Med., Tokyo, Japan; [2]Hoshi Univ. Sch. of Pharm. and Pharm. Scie., Tokyo, Japan.; [3]Faculty of Health Care, Kiryu Univ., Gunma, Japan.*)

Galanin-like peptide (GALP) is well known as a neuropeptide regulating feeding behavior and energy metabolism. In this study, we examined anti-obesity effect of GALP by focusing on lipid metabolism. Mice were i.c.v. injected saline or GALP, and removal of the liver and adipose tissue at 100 minutes after the administration of GALP. Then, we studied hepatic and adipose tissue lipid metabolism related gene expression by use of real-time PCR analysis. Next, to investigate the anti-obesity effect of chronic administration of GALP, mice were fed a high fat diet to induce obesity and were intranasal administrated of GALP for 2 week. The respiratory exchange ratio of GALP group was lower than that of the saline group. In the GALP group, fatty acid oxidation-related gene mRNA levels were increased in the liver. In the adipose tissue, the mRNA levels of HSL and ATGL, were increased in the GALP group. In chronic infusion study, the body weight gain was decreased by GALP treatment as compared with the control group. Hepatic triglyceride levels decreased and, fatty acid oxidation-related genes expression were increased in GALP group. The present study indicates that GALP stimulates the hepatic lipid metabolism and anti-obese effect of GALP may be caused by improvement of lipid metabolism in the liver. It is thought that GALP may be effective for treatment and the prevention for obesity and life-style-related diseases in the near future.

(COI: No)

S71-2

Paraventricular nucleus NUCB2/nesfatin-1 neuron is targeted by leptin and regulates feeding

Nakata, Masanori; Darambazar, Gantulga; Wang, Lei; Yada, Toshihiko (*Dept. Physiology, Division of Integrative Physiology, Jichi Medical University*)

Nesfatin-1, an anorectic peptide processed from nucleobindin-2 (NUCB2), is expressed in the hypothalamus including the paraventricular nucleus (PVN), the region serving as the integrative center for energy homeostasis. Central and peripheral injections of nesfatin-1 decrease food intake in rats and mice. Accumulating evidences suggest that the NUCB2/nesfatin-1 localized in PVN is an emerging new player in regulation of food intake and energy metabolism. In this study, we used adeno-associated virus (AAV) vectors encoding shRNA targeting NUCB2 (AAV-NUCB2-shRNA) and examined the role of PVN NUCB2/nesfatin-1 in feeding behavior. PVN-specific NUCB2 knockdown resulted in increases in food intake during light phase and body weight gain, without affecting energy expenditure. Moreover, anorexigenic ability of peripherally- and centrally-administered leptin was impaired in mice receiving AAV-NUCB2-shRNA. Leptin markedly increased NUCB2 mRNA expression in PVN in vivo and in vitro. Leptin induced Ca^{+} signaling in PVN NUCB2/nesftin-1-immunoractive neurons. These results demonstrate that the PVN NUCB2/nesfatin-1 physiologically regulates feeding and body weight and serves as the direct target for the anorexigenic action of leptin.

(COI: No)

S71-3

AMPK in the paraventricular hypothalamic nucleus regulates food selection behavior in mice

Okamoto, Shiki (*Endocrinol Metab, NIPS, Aichi, Japan*)

Hypothalamic AMP-kinase (AMPK) regulates feeding behavior in response to hormonal and nutrient signals. However, the effect of AMPK on food preference remains to be established. We found that refeeding after overnight fasting, which activates AMPK in the PVH, increased the selection of high carbohydrate diet (HCD) but decreased that of high fat diet (HFD) in mice. The effect of fasting was suppressed by expression of shRNA for AMPK alpha1 and 2 in the PVH with lenti virus. In contrast, expression of constitutively-active AMPK (CA-AMPK) in PVH neurons increased HCD selection. We examined the principle neurons in the PVH for the regulation of food selection behavior. Microinjection of CRH into the PVH was found to increase HCD selection, and expression of shRNA for CRH in the PVH blunted the HCD selection in response to fasting. Preferential expression of hM3Dq or CA-AMPK in CRH Cre neurons also increased the HCD selection. We found that the change in food selection was dependent on the AMPK-induced fatty acid oxidation (FAO) in the PVH. Furthermore, pharmacological activation of AMPK increased cytosolic [Ca2+] in CRH neurons isolated from the PVH, and the effect of AMPK was abolished with the expression of shRNA for AMPK or with the suppression of FAO. Diet-induced and genetically obese mice have been shown to prefer HFD. We found that the obese mice decreased AMPK and FAO activity as well as CRH mRNA expression in the PVH.Thus, our results suggest that AMPK-FAO system in CRH neurons in the PVH regulates food selection behavior for HCD and HFD.

(COI: No)

S71-4

Neuropeptide W (NPW) induced hypophagia is mediated via CRH neurons

Takenoya, Fumiko[1,2]; Hirako, Satoshi[2]; Wada, Nobuhiro[2]; Kageyama, Haruaki[3]; Shioda, Seiji[2] (*[1]Dept. Ex. Sports. Phys. Hoshi Univ. Sch. Pharm. Sci. Toky, Japan; [2]Dept. Anat. Showa Univ. Sch. Med, Tokyo, Japan.; [3]Dept. Nutrition, Faculty Health Care, Kiryu Univ. Gunma, Japan.*)

Neuropeptide W (NPW), which was isolated from the porcine hypothalamus, which belong to the G protein-coupled receptor family. Centrally administered NPW is known to suppress feeding behaviour and promotes to secret drenocorticotropin hormone (ACTH) and corticosterone. It is reported that NPW is involved in the regulation of the hypothalamus-pituitary-adrenal cortex (HPA) axis. The aim of this study was to ascertain the roles of NPW in feeding regulations axis via CRH neurons. We observed that NPW-containing axon terminals were make synapses on CRH cell bodies and dendritic processes in the PVN. Central infusion of NPW induced c-Fos expression in the PVN compered to saline injection to the mice, but not vasopressin- nor oxytocin-positive neurons in the PVN. To determine whether NPW regulates feeding behaviour via CRH neurones, the feeding behaviour of rats was studied following NPW i.c.v. injection with or without CRH antagonist pretreatment. The CRH antagonist canceled the NPW-induced anorexia. Moreover, using the CellKey system which enables comprehensive pharmacological evaluation of cell surface receptors, including GPCRs and tyrosine kinase receptors, using adherent and suspension cell lines and primary cells and have been shown to be characteristic of Gs, Gq, and Gi GPCRs of analysis. NPW response in cells on CellKey System was Gi axis. These results suggested that NPW mediated neuronal feeding pathway via CRH neurons in the PVN.

(COI: No)

S71-5

Clinical application of GLP-1 to obesity-related diabetes

Ueno, Hiroaki; Nakazato, Masamitsu (*Internal Med., Miyazaki Univ., Miyazaki, Japan*)

Glucagon-like peptide-1 (GLP-1) regulates diverse physiological phenomena such as insulin and glucagon secretion, gut motility, and appetite. We administered GLP-1 and saline during a test meal to healthy subjects and patients with type 2 diabetes to assess the role of GLP-1 in regulating glucose metabolism, gut peptides, appetite, and the autonomic nervous system. In both groups, GLP-1 administration significantly decreased plasma glucose and glucagon levels, and increased insulin and blood pressure. Feelings of fullness and hunger were similar between GLP-1 and saline in both groups. This GLP-1 test may be useful for predicting the effects of incretin-related drugs and assessing insulin secretion capacity; however, further studies are necessary.
We developed a novel device and GLP-1 compound for intranasal administration. Twenty-six patients with type 2 diabetes were enrolled in a double-blind placebo-controlled study. Intranasal GLP-1 or placebo was administered immediately before every meal for 2 weeks. The plasma peak concentration of active GLP-1 was 47.2 pmol/L, and Tmax was 8.1 min. The early phases of insulin and glucagon secretion were recovered and suppressed, respectively, in the GLP-1 group. Glycoalbumin decreased significantly after GLP-1 administration. Body weight and appetite were unchanged. Subjects exhibited no marked adverse events after using nasal GLP-1. Long-term application of the drug, including body weight reduction, should be evaluated in future trials.

(COI: No)

Award Posters of the PSJ

（March 21, 12:45～14:00）

AP-1～AP-2	Promotion Award of the Physiological Society of Japan for Young Scientists
AP-3～AP-7	Hiroshi and Aya Irisawa Memorial Promotion Award for Young Physiologists
AP-8	Hiroshi and Aya Irisawa Memorial Promotion Award for Cardiovascular Physiologists
AP-9	Aya Irisawa Memorial Promotion Award for Excellence by Women Physiologists

AP-1 (P1-206)

TRPM2 protects mice against polymicrobial sepsis by enhancing bacterial clearance

Numata, Tomohiro[1,2,3]; Qian, Xiaowei[4]; Inoue, Ryuji[1]; Fang, Xiangming[4]; Mori, Yasuo[2,3] (*[1]Dept of Physiol, Fukuoka Univ. Sch. of Med, Fukuoka, Japan; [2]Grad Sch of Env Stu, Kyoto Univ, Kyoto, Japan; [3]Grad Sch of Eng, Kyoto Univ, Kyoto, Japan; [4]Col of Med, Zhejiang Univ, Hangzhou, China*)

TRPM2 is an oxidative stress-activated nonselective Ca^{2+} permeable channel abundantly expressed in macrophages to regulate production of inflammatory mediators. However, the role and mechanism of TRPM2 in polymicrobial sepsis remains unclear. Using CLP-induced polymicrobial sepsis model, Trpm2-KO mice had increased mortality compared with wild-type (WT) mice. The increased mortality was associated with increased bacterial burden, organ injury, and systemic inflammation. TRPM2-mediated Ca^{2+} influx plays an important role in LPS or CLP-induced HO-1 expression in macrophage. HO-1 up-regulation decreased bacterial burden both in WT BMDMs and in CLP-induced septic WT mice. Disruption of TRPM2 decreased HO-1 expression and increased bacterial burden in BMDMs. Interestingly, pretreatment of Trpm2-KO BMDMs with HO-1 inducer markedly increased HO-1 expression and decreased bacterial burden. Moreover, pretreatment of Trpm2-KO mice with HO-1 inducer reversed the susceptibility of Trpm2-KO mice to sepsis by enhancing bacterial clearance. In addition, septic patients with lower monocytic TRPM2 and HO-1 mRNA levels had a worse outcome compared with septic patients with normal monocytic TRPM2 and HO-1 mRNA levels. TRPM2 levels correlated with HO-1 levels in septic patients. Our data demonstrate a protective role of TRPM2 in controlling bacterial clearance during polymicrobial sepsis possibly by regulating HO-1 expression.

(COI: No)

AP-2 (P1-067)

Identification of retrograde signals required for synapse elimination in the developing brain

Uesaka, Naofumi; Kano, Masanobu (*Dept. Neurophysiol., Grad Sch Med, Univ of Tokyo, Tokyo, Japan*)

Precise formation of neural circuits during development is a prerequisite for proper brain functions. Neurons form exuberant synapses with target cells early in development. Then, necessary synapses are selectively strengthened whereas unnercessary connections are weakened and eventually eliminated during the course of postnatal development. This process is known as synapse elimination. Synapse elimination is an important step to shape initial redundant neural circuits into functionally mature circuits, and the disruption is likely linked to mental disorder and brain dysfunction. While the underlying mechanism is still unclear in any systems, several lines of evidence suggest that retrograde signaling from postsynaptic cells regulates synapse elimination. However, these retrograde signals remain to be identified. We have screened retrograde molecules required for synapse elimination of climbing fiber to Purkinje cell connection in the developing cerebellum. We identified some key retrograde molecules which strengthen necessary synapses and eliminate unnecessary synapses. Here I am going to talk about the role of these retrograde molecules in synapse elimination.

(COI: No)

AP-3 (P1-012)

Voltage-dependent movement of the catalytic domain of voltage-sensing phosphatase, VSP, probed by the site-specific incorporation of a fluorescent unnatural amino acid

Sakata, Souhei[1,2]; Okamura, Yasushi[1] (*[1]Lab. Integrative Physiol., Grad. Sch. Med., Osaka Univ.; [2]Inst. Academic Initiative, Osaka Univ.*)

Voltage-sensing phosphatase, VSP, consists of a voltage sensor and a cytoplasmic catalytic domain (CD). The enzymatic activity has been shown to be coupled to the voltage sensor movement. It has been proposed that the voltage-sensor activation induces the conformation change of CD. However, the direct evidence has been lacking. To monitor the voltage-dependent conformation change of CD, we genetically incorporated a fluorescent unnatural amino acid, Anap, into CD. First, Anap was incorporated into "gating loop" which has been claimed to make large conformational change for switching enzymatic activity based on the crystallographic study of CD of VSP. Anap fluorescence was changed in a voltage-dependent manner, indicating that CD changes its conformation upon the voltage-sensor activation. Besides the conformation change, it is also possible that the voltage sensor regulates the distance between CD and the plasma membrane. Since the substrate of the enzyme is phosphoinositides which are membrane components, membrane binding of CD may be crucial for the enzymatic activity. To detect the change of the distance between CD and the plasma membrane, CD and the plasma membrane were labeled by Anap and dipicrylamine(DPA), respectively. We verified that DPA works as a FRET acceptor of Anap on VSP and are currently testing if the distance between CD and the plasma membrane is changed during the voltage-dependent phosphatase activity.

(COI: No)

AP-4 (P1-059)

Cancer cell-specific crosstalk between Na^+, K^+-ATPase and volume-sensitive anion channel in membrane microdomains exerts anti-proliferative activity

Fujii, Takuto[1]; Yamamoto, Shota[1]; Funayama, Keisuke[1]; Shimizu, Takahiro[1]; Takeshima, Hiroshi[2]; Sakai, Hideki[1] (*[1]Dept. Pharm. Physiol., Grad. Sch. Med. Pharm. Sci., Univ. Toyama, Toyama, Japan.; [2]Dept. Biol. Chem., Grad. Sch. Pharm. Sci., Kyoto Univ., Kyoto, Japan.*)

Na^+, K^+-ATPase is a potential target for anti-cancer therapy, because cardiac glycosides, inhibitors of Na^+, K^+-ATPase, potently block cancer cell growth. However, the mechanism underlying the anti-cancer effects of cardiac glycosides is not fully understood. In the present study, we found that ouabain, a cardiac glycoside, inhibited cancer cell proliferation via activation of volume-sensitive outwardly rectifying (VSOR) anion channel. The effects were suppressed by DCPIB, a selective inhibitor of VSOR channel, and the knockdown of Na^+, K^+-ATPase α1-isoform (α1NaK) or VSOR channel component LRRC8A (SWELL1). The disruption of membrane microdomains by methyl-β-cyclodextrin and the attenuation of the production of reactive oxygen species (ROS) by the inhibitors of NADPH oxidase (NOX) significantly suppressed the ouabain-induced VSOR activation and inhibition of cell proliferation. On the other hand, the ouabain-induced effects were not observed in non-cancer cells. These results suggest that α1NaK, NOX and VSOR channels form a signalosome in the membrane microdomains of cancer cells, and that the cardiac glycoside exerts anti-cancer activity through the cancer-specific signalosome.

(COI: No)

AP-5 (P2-220)

In vivo assessment of cardiac autonomic nerve activities and identification of cardioprotective agents for heart failure treatment using atrial microdialysis technique

Shimizu, Shuji; Kawada, Toru; Akiyama, Tsuyoshi; Kamiya, Atsunori; Shishido, Toshiaki; Shirai, Mikiyasu; Sugimachi, Masaru (*Natl Cereb Cardiovasc Ctr, Osaka, Japan*)

Introduction: Sympathoexcitation and vagal withdrawal are causes of heart failure progression. Therefore, sympatho-suppression using beta-blockers has been a gold standard treatment for heart failure. We developed the atrial microdialysis technique to simultaneously assess cardiac sympathetic and vagal activities. Using this technique, we examined the effects of various pharmacological agents on cardiac autonomic nerve activities to identify cardioprotective agents.

Methods: In anesthetized rabbits, a dialysis probe was implanted into the right atrial myocardium near the sinoatrial node and was perfused by the Ringer's solution. Dialysate norepinephrine (NE) and acetylcholine (ACh) concentrations were analyzed as indices of cardiac autonomic nerve activities using high-performance liquid chromatography.

Results: 1) Electrical stimulation of sympathetic nerve or vagal nerve significantly increased dialysate NE or ACh concentration in a frequency-dependent manner. 2) Intravenous injection of medetomidine or guanfacine significantly increased dialysate ACh concentration. Furthermore, medetomidine significantly suppressed sympathetic NE release. 3) Intracerebroventricular injection of ghrelin significantly enhanced vagal ACh release to the heart.

Conclusions: Atrial microdialysis technique enabled us to simultaneously monitor cardiac sympathetic and vagal nerve activities. This technique may be useful for the identification of cardioprotective agents.

(COI: No)

AP-6 (P1-034)

Molecular mechanism and regulation of partial agonism of the M2 muscarinic receptor-activated K^+ currents

Furutani, Kazuharu; Chen, I-shan; Inanobe, Atsushi; Kurachi, Yoshihisa (*Dept Pharmacol, Grad Sch Med, Osaka Univ, Osaka, Japan*)

Partial agonists are clinically used to avoid overstimulation of receptor-mediated signaling, as they produce a submaximal response even at 100% receptor occupancy. In addition to signaling activators, several regulators help control intracellular signal transductions. However, it remains unclear whether these signaling regulators contribute to partial agonism. Here we show that regulator of G-protein signaling (RGS) 4 is a determinant for partial agonism of the M2 muscarinic receptor (M2R). In rat atrial myocytes, pilocarpine evoked smaller G-protein-gated K^+ inwardly rectifying (K_G) currents than that evoked by ACh. In a *Xenopus* oocyte expression system, pilocarpine acted as a partial agonist in the presence of RGS4 as it did in atrial myocytes, while it acted like a full agonist in the absence of RGS4. Functional couplings within agonist-receptor complex/G-protein/RGS system controlled the efficacy of pilocarpine relative to ACh. Pilocarpine-M2R complex suppressed G-protein-mediated activation of K_G currents via RGS4. Such RGS4-mediated regulation was enhanced at hyperpolarized potentials. We also found that the relative efficacy of pilocarpine to ACh changed upon membrane voltages. Our results demonstrate that partial agonism of M2R is regulated by the RGS4-mediated inhibition of G-protein signaling. This finding helps us to understand the molecular components and mechanism underling the partial agonism of M2R-mediated physiological responses.

(COI: No)

AP-7 (P2-217)

Rapid cholinergic and delayed β-adrenergic vasodilatation in non-contracting muscles during one-armed cranking

Ishii, Kei[1]; Matsukawa, Kanji[1]; Liang, Nan[1]; Endo, Kana[1]; Idesako, Mitsuhiro[1]; Hamada, Hironobu[2]; Kataoka, Tsuyoshi[3]; Yamashita, Kaori[3]; Watanabe, Tae[3]
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We have reported that the rapid cholinergic and delayed β-adrenergic vasodilatation increases blood flow of non-contracting vastus lateralis (VL) muscle during one-legged cycling (Ishii et al. 2013, 2014). It was unclear whether such mechanisms contribute to vasodilatation in non-contracting muscles during one-armed exercise. We examined the influences of atropine and/or propranolol on the blood flow responses of the contralateral biceps and triceps brachii and forearm extensor muscles and VL muscle during moderate one-armed cranking for 1 min (n=7). As an index of muscle tissue blood flow, relative concentration in oxygenated-hemoglobin (Oxy-Hb) was measured using near-infrared spectroscopy. The Oxy-Hb of the muscles increased during one-armed cranking. The increase in Oxy-Hb at the early period of exercise was blunted by atropine, whereas propranolol attenuated the later increase in Oxy-Hb during the exercise. Following combined atropine and propranolol, the Oxy-Hb decreased during the exercise. The influences of the autonomic blockades on the Oxy-Hb response were not different among the muscles. It was concluded that the rapid cholinergic and delayed β-adrenergic vasodilatation increased the blood flows of non-contracting arm and leg muscles during one-armed exercise.

(COI: No)

AP-8

Discharges of aortic and carotid sinus baroreceptors during spontaneous motor activity- and pharmacologically-evoked pressor interventions

Matsukawa, Kanji; Ishii, Kei; Kadowaki, Akito; Ishida, Tomoko; Idesako, Mitsuhiro; Liang, Nan (*Dept Integrative Physiol, Grad Sch Biomed Health Sci, Hiroshima Univ, Hiroshima, Japan*)

We have shown that the cardiomotor component of aortic baroreflex is inhibited at onset of spontaneous motor activity in decerebrate cats, without altering carotid sinus baroreflex. The dissociation may be attributed to a difference in the responses between aortic nerve activity (AoNA) and carotid sinus nerve activity (CsNA). The stimulus-response curves of mean AoNA and CsNA per beat against mean arterial blood pressure (MAP) were compared between the pressor interventions evoked by spontaneous motor activity and by iv injection of phenylephrine or norepinephrine, in which the responses in heart rate (HR) were opposite (i.e., tachycardia vs. baroreflex bradycardia) despite the identical increase in MAP. The stimulus-response curves of the AoNA and CsNA matched between spontaneous motor activity- and pharmacologically-evoked pressor intervention and the slopes of the relative percent AoNA and CsNA were equal between the two interventions. Diastolic AoNA and CsNA (defined as the minimal value within a beat) during spontaneous motor activity showed a greater increase in association with tachycardia, which was abolished by fixing HR at the intrinsic cardiac frequency. Thus mean mass activities of aortic and carotid sinus baroreceptors can faithfully encode the beat-by-beat changes in MAP not only at rest but also during spontaneous motor activity and cannot explain the spontaneous motor activity-related inhibition of the cardiomotor component of aortic baroreflex.

(COI: No)

AP-9

Mechanism of thalamic network remodeling after the peripheral nerve injury

Miyata, Mariko (*Dept Physiol, Sch Med, Tokyo Women's Medical Univ*)

Peripheral sensory nerve injury causes large-scale somatotopic reorganization in the brain. However, neural circuit mechanisms by which the reorganization occurs remain largely unknown. A relay neuron in the mouse whisker sensory thalamus (V2 VPm) receives generally a single afferent fiber originating from the whisker-representing trigeminal nucleus (PrV2). We here found that this one-to-one synaptic relationship was disrupted within one week after transection of the whisker sensory nerve: newly afferent fibers were recruited onto a relay neuron after the nerve transection. Using the Krox20-Ai14 transgenic mouse, in which PrV2-origin afferent fibers are specifically labeled with fluorescent protein, we found that non-PrV2-origin afferent terminals significantly increased in the V2 VPm after the injury, whereas PrV2-origin afferent terminals decreased and weakened around the same time as the synaptic remodeling. Origins of non-PrV2-origin afferent fibers after the transection included the mandibular (V3) subregions of trigeminal nuclei and the dorsal column nuclei, which normally represent body parts other than whiskers. These results indicate that the transection of whisker sensory nerve induces considerable retraction of PrV2-origin afferent fibers and invasion of non-PrV2-origin ones in the V2 VPm, thereby induces large-scale somatotopic reorganization.

We also found tonic $GABA_A$ receptor current was potentiated much earlier than the remodeling of lemniscal fibers onto VPM neurons. Moreover, we found that chronic infusion of tonic GABA agonist into the VPM of normal mice could recruit additional lemniscal fibers onto VPM neurons, whereas lack of tonic $GABA_A$ receptor currents prevented the remodeling of lemniscal fibers after the IONC. These results indicate that enhancement of tonic $GABA_A$ receptor current was crucial for the remodeling of lemniscal fibers after the injury.

(COI: No)

Poster Presentations

Day 1

（March 21, 12:45～14:00）

P1-001～P1-060	Ion channels, Receptors
P1-061～P1-124	Neurons, Synapses
P1-125～P1-157	Molecular anatomy, Molecular physiology
P1-158～P1-197	Organelle, Membrane transport
P1-198～P1-209	Others of Molecular anatomy, Molecular physiology, Cell biology
P1-210～P1-234	Experimental methods
P1-235～P1-355	Undergraduate Poster Presentations

P1-001

Involvement of extracellular Ca^{2+} in the heat-evoked activation of green anole TRPA1

Kurganov, Erkin[1,2]; Tominaga, Makoto[1,2,3] (*[1]Division of Cell Signaling, NIPS, Okazaki, Japan; [2]The Graduate University for Advanced Studies, SOKENDAI; [3]Okazaki Institute for Integrative Bioscience, Okazaki, Japan*)

Transient receptor potential ankyrin 1 (TRPA1) is a Ca^{2+}-permeable nonselective cation channel expressed in nociceptors and activated by irritant compounds such as allyl isothiocyanate (AITC) and temperature. TRPA1 was initially identified as a potential mediator of noxious cold stimuli in rodent nociceptive sensory neurons while TRPA1s from non-mammalian vertebrates (snakes, green anole lizards, frogs and chickens) were recently reported to be activated by heat, but not cold stimulus. A number of studies have shown that intracellular Ca^{2+} is a key regulator of many TRP channels, including TRPA1. In the previous study, we found that extracellular Ca^{2+}, but not intracellular Ca^{2+} plays an important role for heat-evoked activation of green anole TRPA1 (gaTRPA1). In this study, we focus on extracellular Ca^{2+}-dependent heat sensitivity of gaTRPA1 by comparing gaTRPA1 with other heat-activated TRPA1s from rat snake and chicken. It was found that, rat snake and chicken TRPA1s are activated by heat with small inward and large outward currents in the absence of extracellular Ca^{2+}. These results suggest that gaTRPA1 channel, but not TRPA1s of rat snake and chicken needs extracellular Ca^{2+} for heat-evoked activation.

(COI: No)

P1-002

Annexin A2 is a modulator of maxi-anion channel (Maxi-Cl) in mouse mammary C127 cells

Islam, Md. Rafiqul[1]; Okada, Toshiaki[1]; Merzlyak, Petr[1,2]; Sabirov, Ravshan Z.[1,2]; Okada, Yasunobu[3] (*[1]Dept. Cell Physiol., Natl. Inst. Physiol. Sci., Okazaki, Japan; [2]Lab. Mol. Physiol., Inst. Bioorgan. Chem., Uzb. Acad. Sci., Tashkent, Uzbekistan; [3]Grad. Univ. Adv. Studies (SOKENDAI), Hayama, Japan*)

The maxi-anion channel (Maxi-Cl) is ubiquitously expressed in mammalian cells and has been reported as an important gateway for release of the anionic signaling molecules, ATP and excitatory amino acids, from cells subjected to osmotic perturbation, ischemia or hypoxia. The molecular nature of this physiologically and pathophysiologically significant channel is yet to be discovered, although its biophysical and pharmacological properties have been well characterized. As a part of our Maxi-Cl molecule identification efforts we have already excluded several genes (such as *Panx1, Panx2, Cx43*) from the candidates (*Am J Physiol Cell Physiol* 2012, 303: C924-C935). In the present study, we investigated a possible relation of Annexin A2 to Maxi-Cl, because its mRNA (*Anxa2*) showed differential expression between Maxi-Cl-rich C127 cells and Maxi-Cl-deficient C1300 cells as judged by microarray analysis. Using both siRNA- and miRNA-mediated transient gene knockdown strategies, we found that Maxi-Cl currents recorded in the inside-out patch-clamp mode were significantly lower in *Anxa2* -silenced C127 cells than in the mock-transfected cells. However, when Annexin A2 was heterologously overexpressed in C1300 cells, the Maxi-Cl activity could not be rescued. Thus, we conclude that Annexin A2 is a modulator but not the molecule itself of Maxi-Cl.

(COI: No)

P1-003

TRPV2 is critical for the maintenance of brown adipose tissue structure and thermogenesis in mice

Sun, Wuping[1]; Uchida, Kunitoshi[1,2]; Takahashi, Nobuyuki[3]; Suzuki, Yoshiro[1,2]; Zhou, Yiming[1]; Takayama, Yasunori[1]; Kawada, Teruo[3]; Wakabayashi, Shigeo[4]; Tominaga, Makoto[1,2] (*[1]Div of Cell Signaling, OIIB (NIPS), Okazaki, Japan; [2]Dep of Physiol Sci, SOKENDAI, Okazaki, Japan; [3]Div of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan; [4]Dep of Molecular Physiology, National Cerebral and Cardiovascular Center, Suita, Osaka, Japan*)

Transient receptor potential vanilloid 2 (TRPV2) is a Ca^{2+}-permeable non-selective cation channel, which plays vital roles in the regulation of various cellular functions. However, the molecular identity and function of TRPV2 remains unexplored in mouse brown adipose tissue (BAT). This study aimed to clarify the expression and function of TRPV2 in brown adipocytes. We found that functional TRPV2 was predominantly expressed in brown adipocytes. Moreover, mRNA levels of multiple genes involved in the mitochondrial oxidative metabolism were significantly lower in TRPV2 knockout (TRPV2KO) mice than wild type (WT) mice. In addition, white adipose tissue weight was significantly larger in TRPV2KO mice than in WT mice, but food intake was not different between these two genotypes. TRPV2KO BAT showed increased sizes of brown adipocytes. And TRPV2KO mice also showed cold intolerance and reduced responses to a β3-adrenergic receptor agonist, BRL37344 administration. In conclusion, TRPV2 is functionally expressed in brown adipocytes. BAT thermogenesis is impaired in TRPV2KO mice. And TRPV2 is critical for the maintainence of BAT structure and thermogensis.

(COI: No)

P1-004

Regulation of CALHM1 ion channel by N-linked glycosylation

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Calcium homeostasis modulator 1 (CALHM1) was identified as a gene linked to the pathogenesis of late-onset Alzheimer's disease. Recent studies have established CALHM1 as a pore-forming subunit of a voltage-gated ion channel, determined structure and function of CALHM1 channel, and revealed its important physiological roles. However, lacking is knowledge about other modes of regulation of CALHM1 channel including ones mediated by post-translational modifications (PMTs). When heterologously expressed, mouse CALHM1 (mCALHM1) immunosignals in western blotting appeared at three positions with one band at the mass of a mCALHM1 monomer, 37 kDa, and two additional bands at larger molecular weights, suggesting that mCALHM1 acquires some sort of PMTs. Peptide-N-glycosidase F shifted the two additional bands to 37 kDa, demonstrating that mCALHM1 is glycosylated at Asn residue(s). While mCALHM1 possesses two predicted N-glycosylation sites, point mutation studies identified a conserved Asn139 as the sole N-glycosylation site. A difference in sensitivity to endoglycosidase H of the two glycosylated forms of mCALHM1 revealed that the acquired N-linked glycan undergoes processing/conversion from high-mannose type to complex type. In the present study, we examined roles of acquisition and processing/conversion of the N-linked glycan on channel function and subcellular localization of mCALHM1 by means of chemical and enzymatic reagents and genetic mutations. Our data provide insights into a novel regulation of CALHM1 channel via N-linked glycosylation.

(COI: No)

P1-005

Oxidative stress-induced inhibition of TRPM7 is resulted from enhancement of its sensitivity to intracellular Mg^{2+}

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We recently reported that TRPM7 current was inhibited by oxidative stress [1]. Whole-cell recordings in WT revealed that TRPM7 current was inhibited by oxidative stress induced by hydrogen peroxide (H_2O_2, 500 μM) in a $[Mg^{2+}]_i$ dependent manner. The TRPM7 current was inhibited by intracellular free Mg^{2+} ($IC_{50(1)}$ 6.5 μM and $IC_{50(2)}$ 467.2 μM), and oxidative stress augmented this inhibition ($IC_{50(1)}$ 1.5 μM and $IC_{50(2)}$ 27.6 μM). In the present study, we further explored the mechanisms for the inhibition by introducing mutations in TRPM7. Tetracycline-inducible HEK cell lines for stably expressing wild type (WT), phosphomimetic mutant (S1107E), and kinase inactive mutant (K1645R) murine TRPM7 were established. The current of S1107E was insensitive to $[Mg^{2+}]_i$ as reported earlier [2], and was not inhibited by H_2O_2 even in the presence of 217 μM $[Mg^{2+}]_i$ (141 ± 12 pA/pF and 124 ± 13 pA/pF, before and after an application of H_2O_2, respectively), which is consistent with our hypothesis that oxidative stress inhibits TRPM7 current by sensitizing the channel to $[Mg^{2+}]_i$. TRPM7 kinase activity does not seem to be involved in the phosphorylation of S1107, because K1645R exhibited similar $[Mg^{2+}]_i$ dependent inhibition by H_2O_2 to that observed in WT. It is suggested that phosphorylation of S1107 by an unidentified kinase other than TRPM7 confers tolerance to oxidative stress-induced current inhibition. [1] Inoue et al., Free Radical Biology & Medicine 72 (2014) 257[2] Hofmann et al., Pflugers Archiv DOI 10.1007/s00424-014-1488-0

(COI: No)

P1-006

Insights into the Gating Regulation Mechanisms of the KcsA Potassium Channel from the Measurements of the Single-Molecule Dynamics

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The KcsA potassium channel is a pH gated channel which keeps the closed state at neutral pH and undergoes gating at acidic pH. To reveal the dynamic feature of the gating, we have engaged in developing the Diffracted X-ray Tracking (DXT) method in which conformational changes of the single channel molecules were recorded as a movie. At acidic pH the channel exhibited large twisting conformational changes and vigorous structural fluctuations. On the other hand, only small fluctuations were found at neutral pH. To examine the relationship between structural fluctuations and the status of the channel, the solution pH was jumped from neutral to acidic by using the caged proton, and the opening conformational changes of the activation gate were measured. The structural fluctuation was enhanced immediately after the pH jump, and the twisting conformational change was initiated after a delay. This enhanced fluctuation was a similar degree to that observed in equilibrium acidic conditions. In addition, the open channel blocker, TBA (tetrabutylammonium), dramatically suppressed the fluctuations and stopped the twisting motions at acidic pH. These results suggest that the level of fluctuations is closely related to the regulations of the gating.

(COI: No)

P1-007

Acidic amino acids near and in Transient Receptor Potential (TRP) domain of TRPM4 channel are required for maintaining its normal Ca^{2+}-sensitivity

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Transient receptor potential melastatin 4 (TRPM4) channel is a Ca^{2+}-activated non-selective cation channel. The Ca^{2+} sensitivity is modulated by calmodulin and PI(4, 5)P_2. However, unidentified intrinsic divalent cation binding sites in TPRM4 have been presumed to exist because the Ca^{2+} sensitivity has not been abolished by the deletions of its calmodulin binding sites. The purposes of this study are to reveal the properties of the divalent cation binding sites in TRPM4 and to identify the possible amino acid residues which form the binding sites. We firstly examined the effects of divalent cations applied to the cytosolic side of the channel by using an inside-out mode patch-clamp technique. Co^{2+}, Mn^{2+}, and Ni^{2+} potentiated TRPM4 currents, but they did not evoke any currents without Ca^{2+}. These data suggest that there are at least two functionally different binding sites: one is a relatively Ca^{2+}-specific binding site and the other is a binding site for Co^{2+}, Mn^{2+}, and Ni^{2+}. Next, we explored amino acid residues responsible for the activation by Ca^{2+} using single amino acid mutagenesis. Mutations of acidic amino acids near and in the TRP domain, which are conserved in TRPM2, M5, and M8, decreased the Ca^{2+} sensitivity but hardly affected the sensitivities for Co^{2+} and PI(4, 5)P_2. These results suggest a novel role of the TRP domain in TRPM4 as a site responsible for maintaining its normal Ca^{2+} sensitivity.

(COI: No)

P1-008

Omega-3 fatty acids activate Slo1 BK channels and lower blood pressure

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Long-chain omega-3 fatty acids such as docosahexaenoic acid (DHA) found in oily fish may offer various health benefits but the underling mechanisms are only poorly understood. In vascular smooth muscle cells, large-conductance $Ca2^{2+}$- and voltage-dependent K^+ (Slo1 BK) channels provide a vasodilatory influence. We found that DHA with EC50 of ~500 nM directly and reversibly activates BK channels composed of the pore-forming Slo1 subunit and the auxiliary subunit $\beta 1$ in excised-patches, increasing currents by up to ~20-fold. The DHA action does not require voltage-sensor activation or $Ca2^{2+}$ binding but depends on an electrostatic interaction within $\beta 1$ or $\beta 4$. DHA acutely lowers blood pressure in anesthetized wild-type but not in Slo1 knockout mice. DHA ethyl ester (DHA EE), found in dietary supplements, fails to activate BK channels and antagonizes the stimulatory effect of DHA. On an equimolar basis, the stimulatory effect DHA on Slo+1 channels was greater than that of eicosapentaenoic acid, alpha-linolenic acid, arachidonic acid, or linoleic acid. Slo1 BK channels are thus receptors for long-chain omega-3 fatty acids that, unlike their ethyl ester derivatives, activate the channels and lower blood pressure.

(COI: No)

P1-009

Distribution of ASIC4 channel in the mouse brain

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Acid-sensing ion channels (ASICs) are neuronal proton-gated cation channels expressed in mammalian central and peripheral nervous systems. Four ASIC genes (from Accn1 to Accn4) and at least six proteins (ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, and ASIC4) have been identified in mammalian organisms so far. In the brain, ASIC1a, ASIC2a and ASIC2b are known to be involved in a wide variety of physiopathological processes related to extracellular pH fluctuation, such as synaptic transmission and ischemic neuronal injury. However, the regional distribution and function of ASIC4 in the brain are largely unknown. To clarify them, we first performed in situ hybridization experiments using a specific cRNA probe for ASIC4. It revealed that ASIC4 transcripts were broadly expressed throughout the brain. The strong expression was observed in the olfactory bulb, piriform cortex, striatum and superior and inferior colliculi, but was hardly detected in the hippocampal structures, which is in disagreement with the related data from the Allen Brain Atlas. We then generated novel ASIC4 reporter mice using the *ASIC4*$^{tm1a(KOMP)mbp}$ targeting vector [the Knockout Mouse Project (KOMP) repository], and confirmed the proper homologous recombination by RT-PCR and Southern blot analysis. We are currently investigating the detailed distribution of ASIC4 transcripts in the brain.

(COI: No)

P1-010

Voltage-dependency of FMRFamide-gated Na^+ channels

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FMRFamide-gated Na^+ channel (FaNaC) is a peptide-gated sodium channel in the ENaC/DEG family. FaNaC is a homo-trimer, and a subunit has two transmembrane domains (M1 and M2). Although the crystallographic structure of FaNaC is not known, homology modeling based on the structure of an acid-sensing ion channel shows that N-terminal region of M2 (pre-M2) constructs the external vestibule of the channel pore. In the vestibule, there are two aspartate residues (D552, D556) which make negative-rings around the central axis of the pore. We previously showed that *Aplysia* FaNaC (AkFaNaC) expressed in *Xenopus* oocytes is inhibited by external Ca^{2+}, and that aspartate residues in pre-M2 (D552) are involved in the Ca^{2+} action (Pflugers Arch, 451:646-656, 2006; Zool Sci, 27:440-448, 2010). We also showed that D552 is a determinant of the inwardly rectifying I-V relationship of FaNaC (J Physiol Sci, 64:141-150, 2014). To examine the permeation properties as well as the gating characteristics of FaNaC, we employed a cut-open oocyte voltage clamp. We found that the gating of FaNaC is voltage dependent, showing an inward relaxation with two time constants in response to a hyperpolarizing voltage step. Relative amplitudes of exponentially increasing components but not time constants were dependent on the membrane potential as well as the concentration of Na^+. The relaxation was also affected by external Ca^{2+}, and was abolished in Ca^{2+}-free solution. We are currently examining whether the position 552 or 556 is involved in the voltage-dependency of FaNaC.

(COI: No)

P1-011

Hydrophobic layer prevents the proton permeation of voltage-gated proton channel

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Voltage-gated proton channel (Hv1/VSOP) has a voltage sensor domain (VSD), which is similar to the VSD (S1-S4) of classical voltage-gated ion channel, but lacks a pore domain (S5-S6). It has an ability of proton permeation through the VSD controlled by membrane voltage and pH in phagocytes and spermatozoa. We have recently revealed the X-ray crystal structure of Hv1/VSOP (Takeshita et al. 2014) and found a hydrophobic layer, which consisted of four hydrophobic residues (V112/S1, L143/S2, L185/S3 and L197/S4). These residues are not conserved in other voltage-sensor proteins. This hydrophobic layer might be important for breaking water wire and thus prevent the proton permeation in closed state. To test this idea, we analyzed the electrophysiological properties of mouse Hv1/VSOP with mutation in the hydrophobic layer heterologously expressed in HEK293T using whole cell patch clamp recording. The proton currents of three cysteine mutants at V112, L143 and L185 were similar to the proton currents of the wild-type. The cysteine mutant of L197 showed a significant change: The inward current was observed at negative voltage. The pH sensitivity and zinc dependence of gating verified that the inward current at negative voltage was proton current through the mutant proteins. These results support the idea that the hydrophobic layer serves as the barrier for proton permeation in closed state.

(COI: No)

P1-012 (AP-3)

Voltage-dependent movement of the catalytic domain of voltage-sensing phosphatase, VSP, probed by the site-specific incorporation of a fluorescent unnatural amino acid

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Voltage-sensing phosphatase, VSP, consists of a voltage sensor and a cytoplasmic catalytic domain (CD). The enzymatic activity has been shown to be coupled to the voltage sensor movement. It has been proposed that the voltage-sensor activation induces the conformation change of CD. However, the direct evidence has been lacking. To monitor the voltage-dependent conformation change of CD, we genetically incorporated a fluorescent unnatural amino acid, Anap, into CD. First, Anap was incorporated into "gating loop" which has been claimed to make large conformational change for switching enzymatic activity based on the crystallographic study of CD of VSP. Anap fluorescence was changed in a voltage-dependent manner, indicating that CD changes its conformation upon the voltage-sensor activation. Besides the conformation change, it is also possible that the voltage sensor regulates the distance between CD and the plasma membrane. Since the substrate of the enzyme is phosphoinositides which are membrane components, membrane binding of CD may be crucial for the enzymatic activity. To detect the change of the distance between CD and the plasma membrane, CD and the plasma membrane were labeled by Anap and dipicrylamine(DPA), respectively. We verified that DPA works as a FRET acceptor of Anap on VSP and are currently testing if the distance between CD and the plasma membrane is changed during the voltage-dependent phosphatase activity.

(COI: No)

P1-013

Identification of nuclear adrenergic receptor in mouse dental blast cells

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Adrenaline circulates throughout the body and shows various physiological functions by acting on its receptors in the plasma membrane of cells and regulating the intracellular signaling cascades from cAMP, IP3 or Ca^{2+}. We happened to detect the localization of β-adrenergic receptor within the nucleus of the mouse dental blast cells. Adrenaline, which is not membrane-permeable, would need a route from the extracellular fluid through the cytoplasm to reach the nuclear receptor. To address this problem, we employed a series of biochemical and physiological investigations. By modified Falck-Hillarp histochemistry, we detected the monoamine signals in the cytoplasmic and nuclear spaces of these cells. Live observation of the lipophilic dye uptake revealed that these cells showed continuous endocytotic activity, which might be an access of adrenaline to its nuclear receptor. We hereby report on the possible regulatory mechanism of nuclear adrenergic receptor activation.

(COI: No)

P1-014

Association of a nicotinic receptor gene polymorphism with spontaneous eyeblink rates

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Spontaneous eyeblink rates greatly vary among individuals from several blinks to a few dozen blinks per minute. Because dopamine agonists immediately increase the blink rate, individual differences in blink rate are used as a behavioral index of central dopamine functioning. However, an association of the blink rate with polymorphisms in dopamine-related genes has yet not been found. In this study, we demonstrated that a genetic variation of the nicotinic acetylcholine receptor CHRNA4 (rs1044396) increased the blink rate while watching a video. A receiver operating characteristic analysis revealed that the blink rate predicts a genetic variation in the nicotinic receptor gene with a significant discrimination level (0.66, p=0.004). The present study suggests that an increased sensitivity to acetylcholine because of the genetic variation of the nicotinic receptor induces dopamine production by the midbrain dopaminergic neurons, resulting in an increased spontaneous eye blink rate.

(COI: No)

P1-015

TRPV1 and Nav1.8 channels expressions in nodose ganglion neurons of rats treated with capsaicin during the neonatal period

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We examined immunohistochemically the expressions of transient receptor potential vanilloid 1 (TRPV1) and Nav1.8, a tetrodotoxin-resistant voltage-gated sodium channel, in vagal primary afferent neurons in nodose ganglion (NG) of rats, which had been treated with capsaicin during neonatal period. The NG was dissociated from deeply anesthetized 6-week-old rats, which had received normal saline (CONT) or capsaicin (50mg/kg) (EXP) intraperitoneally a day after birth. The frozen serial sections were incubated with primary antibodies directed against TRPV1 and Nav1.8 channels and then double-stained with secondary antibodies. Immunofluorescence was visualized by using a spectral confocal microscope. Total numbers of neurons (per section) in CONT and EXP groups were 96 and 21, respectively, and proportion of the neurons, which showed detectable expression of TRPV1 was 47 % (CONT) and 72 % (EXP) and for Nav1.8 it was 59 % (CONT) and 49 % (EXP). In addition, 32 % (CONT) and 40 % (EXP) of neurons showed co-expression of TRPV1 and Nav1.8. Our results suggest that physiological alterations occurs in vagal afferents after neonatal capsaicin treatment, which is applied to eliminate TRPV1-expressing neurons, may involves effects related to Nav1.8 channels, which are highly co-expressed with TRPV1 channels in NG neurons and therefore its expression could be influenced by neonatal capsaicin treatment.

(COI: No)

P1-016

mASIC4 is a Zn^{2+}-sensitive constitutively active channel

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Acid-sensing ion channels (ASICs) are neuronal cation channels activated by extracellular acidification. There are four ASIC genes that encode at least six individual subunits with distinct and overlapping patterns of expression. ASIC1a, ASIC1b, ASIC2a and ASIC3 can be directly activated by external protons, and ASIC2b is a modulatory subunit of the family. However, ASIC4 has not yet been shown to produce or modulate proton-evoked currents. To investigate the electrophysiological properties of mouse ASIC4 (mASIC4), two-electrode voltage-clamp recordings were performed. It was revealed that expression of ASIC4 in Xenopus laevis oocytes generated small inward currents at a holding potential of -60 mV, while there was no substantial current in H2O-injected control oocytes. The ASIC4 current was insensitive to a nonselective ASIC channel blocker, amiloride, but was reversibly inhibited by 100 μM Zn2+ (IC50 = 89 μM, 95% confidence interval: 63-126 μM). The Zn2+-sensitive current was highly selective for Na+ as the reversal potential was approximately 10 mV in NaCl bathing solution (ND96). Interestingly, the ASIC4 leakage current was enhanced in the presence of extracellular protons, and the current was also blocked by 100 μM Zn2+. These results raise the possibility that ASIC4 associates with other ASIC subtypes to form heteromeric assemblies with the novel electrophysiological properties in the mammalian nervous system.

(COI: No)

P1-017

Guaiacol activates TRPV3 channels in mouse odontoblast-lineage cells

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Guaiacol is used for endodontic treatment and has high analgesic activity. Odontoblasts play important roles in nociceptive sensitivity via the activation of transient receptor potential (TRP) channels, that participate in receiving the stimuli at the dentin surface. In this study, to elucidate the pharmacological effects of guaiacol on the activation of Ca^{2+}-permeable channels in mouse odontoblast-lineage cells (OLCs), we examined the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) by fura-2 fluorescence imaging. In the presence of extracellular Ca^{2+}, we could observe a transient increase in $[Ca^{2+}]_i$ upon treatment with 0.9 μM guaiacol; however, this effect was not observed in the absence of extracellular Ca^{2+}. Repeated application of guaiacol elicited a significant desensitizing effect on Ca^{2+} entry. Increase in $[Ca^{2+}]_i$ by guaiacol was not inhibited by the antagonists of TRPV2 or TRPV4 channels, but was slightly inhibited by a TRPV1 antagonist (capsazepine). Moreover, this guaiacol-induced increase in $[Ca^{2+}]_i$ was significantly inhibited by a TRPV3 antagonist, 2, 2-diphenyltetrahydrofuran. Immunoreactivity against TRPV3 was observed in the OLCs. These results indicate that guaiacol evokes Ca^{2+} influx from the extracellular medium via Ca^{2+} channels in OLCs, and the activation of TRPV3 channels on the plasma membrane of odontoblasts is related to this guaiacol-induced Ca^{2+} influx.

(COI: No)

P1-018

X-ray crystal structure of voltage-gated proton channel

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Voltage-gated proton channel, VSOP, has a voltage-sensor domain but lacks an authentic pore domain. VSOP is required for high-level superoxide production coupling with NADPH oxidase and so on. Our crystal structure of mouse VSOP showed a closed umbrella shape with a long helix consisting of the cytoplasmic coiled-coil and the voltage-sensing helix, S4, and featured a wide inner-accessible vestibule. We also found a Zn^{2+} ion at the extracellular region of mVSOP. The binding of Zn^{2+} suggested that the crystal structure represents the resting state, since Zn^{2+} specifically inhibits activities of VSOP. Actually, two out of three arginines as sensor residues (R204 and R207) were located lower than the conserved phenylalanine, F146, on the S2 in a charge transfer center. This makes contrast with previous structures of other VSDs in the activated state where many positive residues of S4 were located upper than the conserved Phe. Additionally, the crystal structure of mVSOP highlighted two hydrophobic barriers. Aspartic acid (D108), which is critical for proton selective permeation, was located facing intracellular vestibule below the inner hydrophobic barrier, thereby being accessible to water from the cytoplasm. Another hydrophobic layer of extracellular side probably ensures interruption of the proton pathway of mHv1 in resting state. These findings provide a novel platform for understanding the general principles of voltage sensing and proton permeation.

(COI: No)

P1-019

The performance of CB1-knockout mice in three-lever task

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Endocannabinoids play a role in synaptic plasticity through activation of presynaptic CB1 receptors. In the basal ganglia, which contribute to motor learning, CB1 receptors are abundantly expressed. So, it is likely that the endocannabinoid system plays a role in motor learning. To test this possibility, we compared the performance between wild-type and CB1-knockout (CB1-KO) mice in the three-lever operant task. In the operant box, three levers were protruded 1.8 cm into the chamber, and the right (A), center (B) and left (C) levers were positioned 2, 4 and 2 cm above the floor, respectively. One training session lasting 60 min was given once a day and five times a week. The mice were trained to press any one of the three levers for a food reward as shaping (one-lever task), and then trained to press three levers in a given sequence (ABC) (three-lever task). After the mice showed good performance of the three-lever task, the order was reversed to CBA (reverse three-lever task). We found that CB1-KO mice displayed normal performance in the three-lever task, but showed some impairment in the one-lever task, and the reverse three-lever task. In the one-lever task, CB1-KO mice showed a delay in increasing the total number of lever press during the first several sessions, and a delay in avoiding the inactive lever after the inactivation of the lever that was pressed most frequently in the previous session. Our data suggested that CB1-KO mice have difficulty in adapting motor action in response to varying demands.

(COI: No)

P1-020

Voltage-dependent single-channel gating kinetics of mouse pannexin 1 channel

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Pannexin 1 (Panx1) channels are anion-selective channels (Ma et al., Pflugers Arch, 2012) that can be activated by mechanical stimuli, membrane depolarization, caspase-mediated cleavage of its C-terminus and interaction with activated P2X7 receptor. Panx1 considered to be a conduit for ATP release, and act as a "find-me" signal that recruits macropharges to apoptic cells and a crucial paracrine regulator of muco-ciliary function in airway epithelia. Although the open probability of mPanx1 channel increases in a voltage-dependent manner, the detailed single-channel gating kinetics of mPanx1 channel is still remains unknown. In this study, we examined the single-channel conductance and opening/closing rates of mouse Panx1 (mPanx1) channel by using the cell-attached mode of the patch-clamp technique. The channel showed an outward rectification of voltage-current relationship with single-channel conductances of ~20 pS at inward current and ~80 pS at outward current. The voltage-dependency of single-channel opening/closing rates are drastically different at hyperpolarized and depolarized conditions in the boundary of the reversal potential. These results suggest that the quantity of movement per unit time and the direction of movement of charge carrier control the voltage-dependency of opening/closing rates of mPanx1 channel.

(COI: No)

P1-021

Fingerprinting catalytic activity of GPCRs on exhaustive set of G protein substrates reveals complex profiles of functional bias

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A defining feature of all known G Protein-Coupled Receptors (GPCRs) is their ability to activate heterotrimeric G proteins. It is thought that much of the GPCR signaling diversity originates from their differential coupling to an array of G protein subunits, whose unique properties underlie distinct cellular responses. However, the rules for how GPCRs engage selective G proteins from their numerous G protein substrates in response to endogenous ligands and synthetic drugs have not been defined. We developed a multi-dimensional platform for comprehensive profiling of GPCR activity on a nearly complete set of all possible G protein substrates in living cells. The key feature of the system includes quantitative analysis of both efficacy and kinetics of G protein activation using reporter-based imaging strategy yielding robust signals with high reproducibility and throughput. We found that individual GPCRs exhibited characteristic, fingerprint-like, profiles of functional selectivity among their G protein substrates varying in timing and magnitude of activation. Furthermore, synthetic agonists and intracellular molecular environment selectively impacted these fingerprints. We propose that fingerprinting individual GPCRs for their G protein preferences using this technology will enhance understanding of GPCR effects on cellular function, higher order physiology and pathophysiology, and needs to be considered in drug development campaigns to better predictive pharmacological effects of synthetic ligands.

(COI: No)

P1-022

The function of Voltage-Sensing Phosphatase in mice sperm

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Voltage-sensing phosphatase (VSP) consists of the voltage-sensor domain and phosphoinositide phosphatase domain. VSP shows phosphatase activity that is coupled to membrane potential (Murata et al, Nature. 2005). While its expression has been known in secondary spermatocyte in testis of mice, its biological function in sperm remains elusive. In the present study, we examined the biological function of VSP in mice sperms, by focusing on acrosomal reaction, capacitation, sperm motility and so on. To elucidate them, we used knockin mice (VSP-KI mice) in that endogenous VSP gene was replaced by Venus for the present study. We neither observed significant difference in the rate of acrosomal reaction nor tyrosine phosphorylation during the capacitation between VSP-KI mice and hetero mice. On the other hand, it appears that sperm from VSP-KI mice shows a different behavior in their motility from hetero and wild type (WT) mice sperm. We also examined whether endogenous VSP can regulate PIP2 levels in mice sperm. A possible mechanism underlying the regulation of sperm function by PIP2 in mice sperms is discussed.

(COI: No)

P1-023

Slow synaptic inward currents in interneurons in the CA3 area of the hippocampus

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Balance of synaptic excitation and inhibition in the hippocampal neuronal circuit plays an important role in memory, cognition, and pathogenesis of neuronal disorders. Metabotropic glutamate receptors (mGluRs) are known to mediate a slow synaptic transmission in the central nervous system (Cosgrove et al., 2011). Many studies reported that genetic disruption or reduced expression of mGluRs was causal for neuronal disorders such as schizophrenia and autism spectrum disorders (Carlisle et al., 2011; Olszewski et al., 2012), indicating that mGluRs might regulate the function of the neuronal circuits. In the CA3 area of the hippocampus interneurons express mGluRs coupled to cationic channels to mediate feedback inhibition onto the CA3 pyramidal cells (Mori et al., 2002). In the present study, we investigated intracellular signaling pathway and channels responsible for slow excitation in CA3 interneurons. So far the preliminary data were obtained as below. Electrical stimulation of the layer of CA3 pyramidal cells evoked an slow inward current (Peak amplitude, 81.13 ± 12.92 pA, n=5) in CA3 interneurons of cultured rat hippocampal slices at a holding potential of -70 mV in the presence of antagonists for AMPA/kainate receptors, NMDA receptors, $GABA_A$ receptors and $GABA_B$ receptors. The slow inward current was abolished by the bath perfusion of Ca^{2+} free solution, indicating that the inward current is of synaptic origin. Inclusion of GDP β s (1 mM) in the patch pipette on the interneuron inhibited the slow inward synaptic current.

(COI: No)

P1-024

mGluR1α-mediated excitation requires G-protein-dependent and Src-ERK1/2-dependent signaling pathways in cerebellar GABAergic interneurons

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In neurons of the various brain areas, stimulation of type I metabotropic glutamate receptors induces slow excitatory responses through the activation of transient receptor potential canonical (TRPC) channels following several signal transduction pathways. In the cerebellar molecular layer interneurons (MLIs), the underlying mechanisms of the mGluR1 α -mediated slow inward current remain unclear. We found that spontaneous firing of mouse cerebellar MLIs was facilitated by mGluR1 α activation, which caused the inward current partially through G-protein activation. Most of the mGluR1 α -mediated inward current was mediated by TRPC1 channels. Nonselective protein tyrosine kinase inhibitors, genistein and AG490, suppressed the mGluR1 α -mediated inward current significantly. Furthermore, the selective Src kinase inhibitor PP2 and the selective extracellular signal-regulated kinase 1/2 (ERK1/2) inhibitors PB98059 and SL327 decreased the inward current significantly. In contrast to cerebellar Purkinje cells, $GABA_B$ receptor activation in MLIs did not alter the mGluR1 α -mediated inward current, suggesting that there is no cross-talk between mGluR1 α and $GABA_B$ receptors in MLIs. Thus, activation of mGluR1 α in MLIs causes the slow excitatory inward current through not only G-protein-dependent but also Src-ERK1/2-dependent signaling pathways.

(COI: No)

P1-025

The kainic acid-induced synchronous oscillation in the rat barrel cortex

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How functional columns are synchronized or desynchronized is an essential problem on columnar integration. It has been reported that bath application of kainic acid induced 1-5 Hz synchronous network oscillation in layer (L2/3) 2/3 of the barrel cortex. We have recently reported that $GABA_B$ receptor-mediated presynaptic inhibition ($GABA_B$-Pre-I) of intracortical inputs was involved in inter-columnar desynchronization in the barrel cortex using dual whole-cell recordings from two L3 pyramidal cells in the mutually adjacent columns. In the present study, we investigated how the $GABA_B$-Pre-I modulates the spatio-temporal patterns of kainic acid-induced network synchronizations in the barrel cortex by using a voltage-sensitive dye imaging method in slice preparations. Bath application of kainic acid caused synchronous oscillations across multiple columns in the barrel cortex. These synchronous oscillations were mainly composed of theta and delta frequency components. An application of $GABA_B$ receptor antagonist, CGP55845, abolished the delta waves, but slightly enhanced the theta waves. These results suggest that the delta waves in the barrel cortex induced by kainic acid are generated by the activity of presynaptic $GABA_B$ receptors expressed in the glutamatergic axon terminals.

(COI: No)

P1-026

Modified autonomic regulation in mice mutated in the β4 subunit of the lh/lh calcium channel

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Genetic analyses have revealed an important association between P/Q-type calcium channel activities and hereditary neurological disorders. The aim of the present study was to elucidate the physiological importance of the β4 subunit with lethargic (lh) mutant mice. The lh mutant had a small thymus with small-to-medium-sized lymphocytes in the medulla. RT-PCR analysis revealed time-dependent changes in the expression levels of the thymus CaV2.1 and β4 subunits. Both the number and size of Purkinje cells were reduced in the lh mouse cerebellum. In addition, immunostaining with anti-CaV2.1 antibody showed that the expression level of the P/Q-type channel-forming CaV2.1 protein was reduced, which may be associated with the ataxic phenotype. ECG analysis showed that the T wave was high in 8-week-old lh mutants; this may be associated with hyperkalemia. Upon pharmacological ECG analysis, 2-3-week-old lh mutants exhibited reduced responses to a beta-blocker and a muscarinic receptor antagonist. Analysis of heart rate variability revealed that the R-R interval was unstable in lh mutants and that both the low- and high-frequency components had increased in extent, indicating that the tonus of both the sympathetic and parasympathetic nervous systems was modified. Thus, our present study revealed that the β4 subunit played a significant role in regulation of sympathetic and parasympathetic nerve activities.

(COI: No)

P1-027

Nerve injury increases expression of alpha-2/delta-1 subunit of L-type calcium channel in the rat dorsal root ganglion

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Immunohistochemistry for alpha-2/delta-1 subunit of L-type calcium channel was performed on the rat dorsal root ganglion (DRG). The immunoreactivity (IR) was detected in half of 4th and 5th lumbar DRG neurons (49%). These neurons were mostly small to medium-sized. In the ganglia, alpha-2/delta-1 subunit -immunoreactive (-IR) neurons with small cell bodies were intensely stained whereas those with medium-sized cell bodies were lightly stained. Transection of the sciatic nerve dramatically increased the number of alpha-2/delta-1 subunit-IR neurons in the DRGs. Sensory neurons mostly expressed alpha-2/delta-1 subunit-IR in the DRGs at 7 days after the nerve transection (85%). The density of alpha-2/delta-1 subunit-IR in DRG neurons was also elevated by the transection. The number of intensely stained neurons with small cell bodies greatly increased in the injured DRGs. Numerous medium-sized and large DRG neurons which were intensely stained appeared after the treatment. In addition, a double immunofluorescence study demonstrated co-expression of alpha-2/delta-1 subunit and c-Jun activating transcription factor 3 in the injured DRGs. These findings indicate that the subunit may be associated with neuropathic pain transmission.

(COI: No)

P1-028

Electrophysiological effects of volatile anesthetic sevoflurane on striatal neurons of mouse

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It has been proposed that volatile anesthetics exert their influence on nervous system by affecting synaptic transmission and excitability of neural membrane. Sevoflurane is a valuable and commonly-used agent for inhalation anesthesia because of its fast action and short recovery time. In this study we examined how the application of sevoflurane affect the excitability of striatal neurons. Electrophysiological recordings were made from medium-sized projection neurons in slices taken from mouse striatum. Bath-applied sevoflurane caused transient depolarization of membrane potential lasting several tens of seconds. The transient depolarization was obscured by blockade of glutamatergic and GABAergic receptors, suggesting the involvement of unusual synaptic transmissions. In fact, previous study demonstrated the transient increase of spontaneous EPSC frequency in this induction period of anesthesia. However prolonged application of sevoflurane seems to decrease the excitability of striatal neurons by enhanced tonic-GABA current and rise in sodium spike threshold. Furthermore, sevoflurane increased input resistance of striatal projection neurons and diminished the voltage deflections in response to hyperpolarizing and depolarizing current pulses. Therefore, the transient excitation followed by continuous inhibition may underlie the effect of sevoflurane.

(COI: No)

P1-029

Cell swelling induced in hypotonic condition causes TRPA1 activation

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Hypotonic solution causes pain sensation in nasal and ocular mucosa. However these molecular mechanisms were not still entirely known. The candidate receptors, which could be activated by hypotonic solution, are Transient Receptor Potential (TRP) channels. Although TRPV4 was reported as a osmotic sensor, this channel was not known any obious roles in sensory nerves. Therefore, the existence of the other candidates is beleaved. In this study, we tried to clarify the ability of TRPA1 to response to the cell swelling, one of physical stress. Here we shows the modulation of TRPA1 activity induced by AITC was occured by hypotonic condition with Ca-imaging method. Moreover cell swelling in hypotonic condition evoked TRPA1 single current in cell-attached mode of patch-clamp experiment when the pippet was attached to plasma membrane after cell swelling, but not before swelling. Furthermore, this single current caused by cell swelling was blocked by known TRPA1 antagonists. Since pre-application of thapsigargin did not inhibit this single current induced by cell swelling, intracellular calcium concentration did not relate to the activation of TRPA1 caused by physical stress. These findings suggest that the cell swelling cause TRPA1 activation in the cells under the hypotonic condition, resulting pain sensation in nasal and ocular mucosa.

(COI: No)

P1-030

Threshold strength of electric fields for orientation of retinal ganglion cell axons *in vitro*

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Electric fields are a predominant guidance cue directing axons of retinal ganglion cells to the future optic disc during embryonic development (Yamashita, 2013). The axons of newborn retinal ganglion cells grow along the extracellular voltage gradient that exists endogenously in the embryonic retina. The extracellular potential is generated by Na^+ transport through epithelial Na^+ channels in retinal neuroepithelial cells. To investigate molecular mechanisms of the electric effect on axon growth, the threshold field strength for axon orientation should be defined in controlled conditions. In the present study, a culture system was built to set up a uniform constant direct current (DC) electric field by continuously measuring a voltage drop between two points in the culture medium, and also by using a negative feedback circuit to regulate supplied currents. A retinal strip (1 mm in width) of a chick embryo was cultured in a constant electric field for 24 hours and the relationship between the direction of axon growth and the field strength was analyzed by staining live axons with fluorescent dye (calcein-AM). The results showed that a voltage gradient of 0.2 mV/mm was enough to direct axons towards the cathode. This field strength is far weaker than the endogenous voltage gradient (15 mV/mm). The present study suggests that the supra-threshold electric field is sufficient for the correct orientation of retinal ganglion cell axons *in vivo*.

Reference:
1) Yamashita, M. Electric axon guidance in embryonic retina: Galvanotropism revisited. ***Biochem. Biophys. Res. Commun.***, 431: 280-283 (2013).

(COI: No)

P1-031

Exploring the original function of AMPA receptor

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AMPA receptor (AMPAR) has been mainly analyzed using adult stage samples in the light of functional importance for higher brain function, such as learning and memory. However, its expression in the immature neuron suggests that AMPAR has different function in early developmental stage from in adult stage. Numbers of subunits have prevented our generating the KO mice and analyzing its contribution to the development. Here, we chose ascidian, which has only one subunit and is suitable for developmental and genetic analysis. Using morpholino oligo knockdown system, decreased AMPAR expression leaded to inhibition of sensory organ formation and developing arrest during metamorphosis. Two types of AMPARs are in the vertebrate, Ca2+ permeable or impermeable, and using both types of receptors as the situation demands enables to establish the mammalian-type learning and memory. We found that ascidian had only Ca2+ permeable AMPAR. We next examined what happened to ascidian when mammalian-like Ca2+ impermeable AMPAR is introduced. Surprisingly such AMPAR did not have channel activity, and the Ca2+ impermeable AMPAR-induced ascidian demonstrated the same abnormalities as AMPAR KD experiments. These results suggest that the evolutionarily-original function of AMPAR might be involved in a specific neuronal morphogenesis and metamorphosis, and advanced learning system using two types of AMPARs could become available only after vertebrate.

(COI: No)

P1-032

Analysis of a new CaV2.1-interacting protein, TNKS2, as a causative protein of Spinocerebellar Ataxia type 6

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Spinocerebellar ataxia type 6 (SCA6) is one of the autosomal dominant cerebellar ataxias. SCA6 is caused by a small expansion of CAG repeats in *CACNA1A*, which encodes the α_{1A} pore-forming subunit of voltage dependent calcium channel, $Ca_V2.1$ (Olga Zhuchenko et al. 1997). When *CACNA1A* is translated, this CAG repeat appears as poly-glutamine (polyQ) chain in carboxyl terminal intracellular region of $Ca_V2.1$. The general mechanism of polyQ diseases is that aggregate of the expanded polyQ protein exerts neurotoxicity. But recent study suggests that at least 54 glutamine resides are needed for aggregate form stably (Martina Stork 2005). Although other polyQ diseases can meet this condition, SCA6 has at most 40 glutamines and cannot meet this condition. However, expanded polyQ chain may form abnormal structure, therefore the interaction of proteins may be changed. To investigate whether the abnormal expansion of polyQ chain changes the interaction between $Ca_V2.1$ and cryptic molecules and exerts neurotoxicity, 2.5×10^6 clones were screened and we discovered a new $Ca_V2.1$-interacting protein, TRF1-interacted ankyrin-related ADP-ribose polymerase 2 (TNKS2 or Tankyrase 2). The interaction between TNKS2 and $Ca_V2.1$ became stronger. In human cells, current density of $Ca_V2.1$ decreased and the expression of $Ca_V2.1$ on the plasma membrane was reduced. All effects were enhanced by abnormal polyQ expansion. Our findings uncover TNKS2 as a key regulator of $Ca_V2.1$ expression on the plasma membrane and as a new SCA6-involving protein.

(COI: No)

P1-033

Regulation of cardiac Cav1.2 channel by redox via modulation of CaM interaction with channel

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Although it has been well documented that redox can modulate Cav1.2 channel activity, the underlying mechanisms are not fully clear. In the present study, we examined the effect of DTT and H_2O_2 on Cav1.2 channel activity using patch-clamp technique in guinea-pig ventricular myocytes and on CaM interaction with Cav1.2 α 1C subunit with pull-down assay. Application of 1 mM DTT in the perfusing solution decreased channel activity to 72%, while H_2O_2 increased channel activity to 303%, suggesting reduction and oxidation played opposite effect in modulation of Cav1.2 channel. Pretreatment of cardiac myocytes with 1 mM DTT and H_2O_2 significantly impact the channel activity induced by CaM (1 μM) + ATP (3 mM) (72% and 176% of control, respectively). To test the hypothesis that redox state might determine channel activity through affecting CaM interaction with the channel, we examined the effect of DTT and H_2O_2 on CaM binding to N- and C-terminal fragment of the channel which contained CaM binding sites. We found that DTT dose-dependently inhibits CaM binding to C-terminus (IC_{50} 37 μM), but H_2O_2 had no effect. Neither DTT nor H_2O_2 had an effect on CaM interaction with N-terminus. These results suggest that redox-mediated regulation of Cav1.2 channel is mediated, at least partially, by modulating CaM interaction with channel.

(COI: No)

P1-034 (AP-6)

Molecular mechanism and regulation of partial agonism of the M2 muscarinic receptor-activated K^+ currents

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Partial agonists are clinically used to avoid overstimulation of receptor-mediated signaling, as they produce a submaximal response even at 100% receptor occupancy. In addition to signaling activators, several regulators help control intracellular signal transductions. However, it remains unclear whether these signaling regulators contribute to partial agonism. Here we show that regulator of G-protein signaling (RGS) 4 is a determinant for partial agonism of the M2 muscarinic receptor (M2R). In rat atrial myocytes, pilocarpine evoked smaller G-protein-gated K^+ inwardly rectifying (K_G) currents than that evoked by ACh. In a *Xenopus* oocyte expression system, pilocarpine acted as a partial agonist in the presence of RGS4 as it did in atrial myocytes, while it acted like a full agonist in the absence of RGS4. Functional couplings within agonist-receptor complex/G-protein/RGS system controlled the efficacy of pilocarpine relative to ACh. Pilocarpine-M2R complex suppressed G-protein-mediated activation of K_G currents via RGS4. Such RGS4-mediated regulation was enhanced at hyperpolarized potentials. We also found that the relative efficacy of pilocarpine to ACh changed upon membrane voltages. Our results demonstrate that partial agonism of M2R is regulated by the RGS4-mediated inhibition of G-protein signaling. This finding helps us to understand the molecular components and mechanism underling the partial agonism of M2R-mediated physiological responses.

(COI: No)

P1-035

Methods for the functional analysis of ion channels on the contact bubble bilayer by the intra-bubble perfusion

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Planar lipid bilayers have been used as the platform for the functional measurement of ion channels. Recently, a stable planar lipid bilayer (droplet interface bilayer, DIB) can be formed easily by contacting two lipid monolayers formed on the surface of small water droplets (ca. 1 mm diameter) in the oil. Here we improve the DIB method for the time-resolved measurement of changes in the electrophysiological properties of ion channels upon instantaneous solution exchange. For this purpose, a minuscule water bubble lined with lipid monolayer is blown from a glass pipette into an oil phase. Two bubbles (each with a diameter of ca. 50 μm) are held side by side to form a bilayer, which is termed a contact bubble bilayer (CBB). The area and curvature of the CBB are under the control of the intra-bubble pressure applied by each pipette. Additionally, the lipid composition of each leaflet of the CBB is readily varied. Because of smaller area of the CBB (ca. 80 μm^2) than the conventional planar lipid bilayers (>1000 μm^2), low background electrical noise in electrophysiological measurements is attained. A rapid perfusion system is also developed by introducing additional pressure-driven injection pipettes to the bubbles. Because the volume of the bubble is small (ca. 300 pL), the solution inside the bubble is exchanged within 20 ms. Example applications of this versatile method are presented to characterize the function of ion channels.

(COI: No)

P1-036

Exploration of temperature sensitivity and new antagonists of acid-sensitive outwardly rectifying anion channel (ASOR)

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It is well known that the acid-sensitive outwardly rectifying anion channel (ASOR), which is expressed in many cell types, is involved in acidotoxic necrotic cell death. However, its physiological significance and molecular identity have not been known yet. In human epithelial HeLa cells and cultured mouse cortical neurons, whole-cell currents of ASOR were augmented by warm temperature with a threshold temperature of 32 ℃ and 26 ℃, respectively. Cell swelling induced by extracellular acidification was inhibited by cooling, and acidosis-induced necrotic cell death was rescued at low temperature in both HeLa cells and cortical neurons. These data indicate that ASOR is sensitive to temperature and that reduced temperature rescues acidotoxic cell death by preventing persistent swelling. In fact, both acidosis-induced cell swelling and necrotic cell death were inhibited by DIDS and phloretin which are known ASOR blockers. We further explored the effects of 13 known antagonists for other types of anion channels, and here found suramin and niflumic acid as new antagonists of ASOR.

(COI: No)

P1-037

Expression Analysis of TRPM7 in Odontoblasts

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Transient receptor potential (TRP) ion channel family is well known to play a role in a sensor such as temperature, osmotic pressure, and redox status. Among TRP channel family, TRPM7 has a unique structure organization that contains a TRP channel domain with 6 transmembrane segments fused to an atypical serine-threonine kinase domain at its C terminus. Genetic deletion of TRPM7 in model systems demonstrates that this channel is critical for cellular growth and embryonic development. In this study, we found that TRPM7 is highly expressed in odontoblasts in the dental pulp by in situ hybridization of mouse embryo. Quantitative real-time PCR analysis revealed that expression of TRPM7 in the tooth was remarkably higher than any other tissue of adult mouse. We also confirmed that TRPM7 protein is expressed in odontoblasts by immunohistochemistry. To investigate the physical function of TRPM7 in odontoblasts, we examined TRPM7 channel activities using a mouse odontoblast cell line. These results suggested that higher expression of TRPM7 play as an important role in odontoblasts. We will also show our recent result of physiological role of TRPM7 in odontoblasts.

(COI: No)

P1-038

Altered voltage sensor movements in KCNQ1 channels with a mutation causing short QT syndrome

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KCNQ1 channel is a cardiac voltage-gated potassium channel. Some of the KCNQ1 mutations can cause short QT syndrome, a rare type of cardiac arrhythmia characterized by shorter depolarization time of cardiac action potential. The mutations for short QT syndrome have been identified only in the S1 and S5 segments and the pore helix. Because the S1 is a part of the voltage-sensing domain (VSD) and the S5 closely interacts with the VSD, we hypothesized that the VSD movement is altered in the short QT mutants in favor of the open state. When the VSD movement was tracked by voltage clamp fluorometry, the wild-type KCNQ1 channel with its auxiliary subunit KCNE1 showed two components of fluorescence rise upon depolarization: The faster component corresponds to the main movement of the VSD (down state to up state) and the slower component relates to the opening of the channel (up state to open state). The short QT mutants in the S1 (S140G and V141M) with KCNE1 showed no faster component, suggesting most of the VSD was in the up state or the open state even at the resting potential. The S5 mutant (I274V) showed a smaller faster component, also indicating the up state was in favor. These results show that the channel is upregulated due to the altered VSD movement in the short QT mutants. Interestingly, the stabilization of the up state and/or the open state was observed only in the presence of KCNE1. The prerequisite of KCNE1 implies that S140, V141 and I274 might serve as one of the interaction sites with KCNE1 for regulation of the VSD movement.

(COI: No)

P1-039

PKA-mediated facilitation of cardiac Cav1.2 channel through uncovering calmodulin binding sites by distal C-terminus of α1c subunit

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PKA-mediated facilitation of Cav1.2 channel plays an essential role in triggering myocytes contraction. However, the underlying mechanism is so far not fully clarified. Our previous studies suggest that CaM interaction determines the channel activity. In the present study, we examined the cross-talk among PKA, calmodulin (CaM), fragments of distal C-terminus of α 1c subunit (CTd) and CaM binding domain peptides (IQ and preIQ) in regulation of Cav1.2 channel in guinea pig ventricular myocytes. 10 μM of cAMP facilitated CaM-induced Ca^{2+} channel activity in the inside-out patches and 10 μM of nonspecific kinase inhibitor, K252a, abolished such a facilitation, suggesting that cAMP-mediated facilitation was through activation of inactive PKA which still attached on the channel in the inside-out patches. Ca^{2+} channel activity in the inside-out patches maintained by 1 μM CaM and 3mM ATP was inhibited by 5 μM of fragment peptide of CTd, while pre-treatment of patches with cAMP, DCT had no effect on CaM-induced channel activity. Furthermore, we found that CaM binding domain peptides, IQ and PreIQ, mimicked cAMP effect and both effects of cAMP and CaM binding domain peptides were not additive. These results suggest that PKA-mediated facilitation of Cav1.2 channel is through uncovering CaM binding sites in the C-terminus.

(COI: No)

P1-040

Mechanism of the complete block of the Kir2.1 inward rectifier K^+ channel under the external K^+-free condition

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Currents through the strong inward rectifier K^+ channels composed of Kir2 subunits are completely blocked under the external K^+-free condition, but the mechanism of this phenomenon remains to be known. We recently reported that this sensitivity to external K^+ is lost in the heterozygous M301K mutation in Kir2.1 (J Physiol Sci, 2014), which is found in the patient exhibiting cardiac and neuronal manifestations. Here, we explored the mechanism underlying the loss of Kir2.1 conductance in the absence of external K^+ using a heterologous mammalian expression system. In whole-cell recordings, Kir2.1/Kir2.1(M301K) heteromeric channels showed a linear outward conductance in the K^+-free external solution. However, K^+ selectivity and $[K^+]_o$ dependence of the conductance were not perturbed. The same findings were obtained with Kir2.1(E224G) and Kir2.1(E299S). Since with all these mutations, negative surface potential inside the cytoplasmic pore is reduced and inward rectification caused by positively-charged polyamines is diminished, we hypothesized that inhibition of Kir2.1 in the absence of external K^+ may be caused by the polyamine block of outward currents. When Kir2.1 currents were obtained from inside-out patches using the external K^+-free pipette solution, the reversal potentials were shifted to a very negative voltage, and outward currents sensitive to polyamines could be recorded. Thus, collapse of Kir2 channel pore does not occur in the absence of external K^+, but outward currents are completely inhibited by polyamine block, which increases with the K^+ driving force.

(COI: No)

P1-041

Zdhhc3/7, the members of protein-palmitoylation enzymes, inhibit the current amplitude of HCN2 channel

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Recent studies revealed that the S-palmitoylation regulates the trafficking and the function of ion channels. Previously, we reported that among hyperpolarization-activated cyclic nucleotide-modulated channel family (HCN1-4), HCN1, HCN2 and HCN4, but not HCN3, are the targets of S-palmitoylation. S-palmitoylation is a reversible post-translational lipid modification; palmitoylation and depalmitoylation are catalyzed by protein acyl transferases (PATs) and palmitoyl protein thioesterases (PPTs), respectively. In this study, we aimed to identify subtypes of these enzymes that regulate the palmitoylation of HCN2 channel. We overexpressed each 24 subtypes of known PATs with HCN2 and found that multiple PATs of Zdhhc-family significantly increased the palmitoylation of HCN2 protein. Especially, 5 Zdhhc proteins (Zdhhc2, Zdhhc3, Zdhhc7, Zdhhc15, and Zdhhc20) augmented the palmitoylation of HCN2 protein approximately over 10-fold of control level. When Zdhhc3 and Zdhhc7 were co-expressed with HCN2 in *Xenopus* oocytes, these enzymes reduced the current amplitude of HCN2 channel, but did not affect voltage-dependent activation of the channel. Therefore, our results suggested that they might regulate the trafficking or the activity of HCN2 on the plasma membrane. Although our results demonstrated that HCN2 channel is the target for certain types of PATs, further studies are indispensable to address whether these enzymes exert physiological roles in the signal transduction system which may regulate HCN channels.

(COI: No)

P1-042

The involvement of TRPM7 in the activity of pancreatic stellate cells

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The activity of pancreatic stellate cells (PSCs) is involved in pancreatic diseases including pancreatitis and cancer. It is known that the conversion of quiescent PSCs to activated PSCs promotes proliferation of PSCs and the development of pancreatic fibrosis and lead to pancreatic diseases. Therefore, it is important to investigate the activity of PSCs for the better understanding of pancreatic diseases. Here, we focused on TRPM7, which is a ubiquitously expressed cationic ion channel. Many reports suggested that TRPM7 is involved in various cellular physiological and pathological processes including differentiation, proliferation and migration. Therefore we hypothesized that TRPM7 is involved in the conversion or proliferation of PSCs. To reveal the contribution of TRPM7 to PSCs, we prepared TRPM7 conditional knockout mouse. First, we confirmed that tamoxifen could induce the deletion of TRPM7 in PSCs in vitro using electrophysiological techniques. Next, we checked whether there are any changes in the expression of TRPM7 that accompany the conversion of PSCs. Then, we revealed that the expression of TRPM7 and α-SMA (marker of PSCs conversion) increased when Platelet-Derived Growth Factor BB (PDGF-BB) induced the conversion of PSCs. These results suggested that TRPM7 may contribute to the conversion of quiescent PSCs to activated PSCs.

(COI: No)

P1-043

Dynamic behavior of the KcsA potassium channel in membrane: Direct observation by high-speed atomic force microscopy

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The KcsA potassium channel is a pH-dependent channel, and the activation gate opens at acidic pH. Using atomic force microscopy (AFM), we have captured the open-gate structure and gating-coupled clustering-dispersion of membrane-embedded KcsA channels by removing the potentially sight-obstructing cytoplasmic domain (CPD). At neutral pH, the closed channels formed self-assembled nanoclusters. At acidic pH, the open-gated channels were dispersed as singly-isolated channels. High-speed AFM revealed that the clustering-dispersion dynamics were reversible and completed within several minutes. Here, further high-speed AFM observation captured channel-channel interaction at sub-molecular resolution. When two open channels engaged in the membrane, they disengaged immediately or fused together to become apparently-closed channels. The interplay between the gating conformational change of individual channels and the clustering-dispersion dynamics provides insight into understanding membrane-mediated protein-protein interactions and functional cooperativity.

(COI: No)

P1-044

Functional analysis of CatSper channel in heterologous expression system

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CatSper channel, a cation channel expressed specifically in testis, is essential for fertilization in mouse. CatSper channel is formed by four alpha subunits, CatSper1, CatSper2, CatSper3 and CatSper4, as a hetero tetramer. Each subunit has six trans-membrane segments (S1-S6). Some features of amino acid sequence, such as positively charged amino acids in the fourth trans-membrane segment (S4) and the acidic residue in selectivity filter in the putative pore forming region of S5-S6, suggest that CatSper is a voltage-dependent calcium channel. However, detailed characteristics of CatSper are still unclear since functional analysis using heterologous expression systems has been unsuccessful. In this study, we report that the region truncated just downstream of S4 of Ci-CatSper3, corresponding to the voltage-sensor domain (VSD) of other voltage-gated ion channels, has calcium permeability. With *Xenopus* oocyte expression system, we detected substantial ionic currents which were activated upon hyperpolarization. Calcium photometry revealed that Ca^{2+} permeable pathway was formed by the truncated form of Ci-CatSper3. Furthermore, introduction of mutations in S4 altered the kinetics of activation, indicating that VSD of Ci-CatSper3 itself has ion permeability. This is, to our knowledge, the first study which reports calcium permeation through CatSper channel in a heterologous expression system.

(COI: No)

P1-045

The C-linker of hERG channel interacts with EAG domain and S4-S5 linker to regulate the slow deactivation

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hERG channel is a member of the voltage-gated K^+ channel (Kv) family, and the main subunit of the rapidly activating delayed rectifier K^+ current (I_{Kr}) in human heart. This channel has six transmembrane segments (S1-S6) consisting of the voltage-sensing domain (VSD, S1-S4) and the pore domain (PD, S5-S6) like other Kv family members, and has rather large intracellular domains. This channel is well known for its slow deactivation, which has been shown to be regulated by an interaction between the intracellular domains such as the EAG domain in the N-terminal cytoplasmic region and the cyclic-nucleotide binding homology domain (CNBHD) in the C-terminal cytoplasmic region. In this study, we newly identified an interaction between the S4-S5 linker (connecting the VSD to the PD) and the C-linker (connecting the PD to the CNBHD) by using site-directed mutagenesis and two-electrode voltage clamp of *Xenopus* oocytes. When glutamic acid residues E544 in the S4-S5 linker and E698-E699 in the C-linker were mutated to lysine (K) residues respectively, deactivation kinetics of E544K mutant and E698K-E699K double mutant were much faster than that of the wild type. Interestingly, E544K-E698K-E699K triple mutant partially rescued the deactivation kinetics. Recently, the interaction between E698-E699 and R4-R5 in the EAG domain has been reported (Ng *et al.*, 2014). Taken together, our and others' results indicate the importance of the interactions among the intracellular domains in the slow deactivation of hERG channel.

(COI: No)

P1-046

Gating of the cytotoxic peptide polytheonamide B channel

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We have examined the channel properties of a cytotoxic peptide, polytheonamide B (pTB), from marine sponge by use of the planar lipid bilayer technique. The pTB channel is a monovalent cation-selective channel as well as the proton permeability. In this study voltage-dependent gating was examined. Macroscopic current of the pTB channel was recorded upon the voltage steps, and the time-dependent activation and deactivation were observed. At pH 1.5, the channel opens at positive potentials, while as the pH becomes more acidic, the channel opens more at negative potentials. The reversal of the voltage-dependent gating has never been reported for any other channels. Our results suggest that protonation of the amino acid residue(s) within the pTB regulates its gating. We will discuss the molecular mechanism underlying proton dependent gating of the pTB channel.

(COI: No)

P1-047

The stoichiometry of Kv4.2/DPP10 complex has a preference to 4:2

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Kv4 is a member of voltage gated K^+ channel family and forms a complex with various auxiliary subunits. Dipeptidyl peptidase-like protein 10 (DPP10) is a membrane protein which has one transmembrane domain. It has been shown that DPP10 increases the current amplitude, accelerates the inactivation and the recovery from inactivation of Kv4. However, it remains unknown how many DPP10s can bind to one Kv4 channel. To test whether or not the expression level of DPP10 can affect the properties of Kv4, Kv4.2 and DPP10 were co-expressed in *Xenopus* oocytes with different ratios. We observed that the electrophysiological properties of Kv4.2, such as time to peak and recovery from inactivation, gradually changed with the increase in DPP10 expression. Before examining the stoichiometry of the Kv4.2/DPP10 complex, we investigated the stoichiometry of DPP10 in the absence of Kv4.2 on the membrane using subunit-counting by single-molecule imaging. We expressed DPP10 tagged with monomeric enhanced green fluorescent protein (DPP10-mEGFP) in *Xenopus* oocytes, and observed that 60%-80% of DPP10s on the membrane exist as dimers. To evaluate the stoichiometry of the Kv4.2/DPP10 complex, we co-expressed Kv4.2-mCherry and DPP10-mEGFP with different ratios (Kv4.2-mCherry: DPP10-mEGFP = 100 : 1, 10 : 1, 1 : 1). In contrast to the Kv4/KChIP complex in our previous study, the stoichiometry data of the Kv4.2/DPP10 complex could not be explained by independent binding to the 4 sites, but it showed a clear preference to 4:2 ratio.

(COI: No)

P1-048

Conserved Trp in the adjacent S4 helices cooperatively produce the sustained-deactivation in the voltage-gated H^+ channel dimer

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The voltage-gated H^+ channel (Hv) is a voltage-sensor domain (VSD) like protein consisting of four transmembrane segments (S1-S4). The molecular structure of Hv is a homo dimer, and each channel subunit functions cooperatively. Here we show that two voltage-sensor S4 helices in dimer cooperate with each other directly via a pi-stacking interaction between two Trp at the middle. Scanning mutagenesis approach showed that the existence of Trp around the original position provided the slow gating kinetics which is a characteristic of the dimer cooperativity. Mutation cycle analysis with the dimeric/monomeric channels suggests that the two Trp in the S4 helices of dimer are energetically-coupled in deactivation but less coupled in activation. Molecular dynamics simulation showed a direct pi-stacking between the two Trp. These results provide a new aspect of the cooperative function of voltage-gated channels in which adjacent voltage-sensor helices have a physical contact and work effectively as a unit in the H^+ homeostasis.

(COI: No)

P1-049

Examination of the role of LRRC8A in the function of volume-sensitive outwardly rectifying anion channel (VSOR)

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Volume-sensitive outwardly rectifying anion channel (VSOR) is one of the volume-regulated anion channels (VRACs) and functionally expressed in almost all cell types. The roles of VSOR in cell volume regulation, proliferation, migration and cell death are well established, but its molecular identity is unclear. In 2014, two research groups independently have reported that LRRC8A is a core factor of VSOR in human cells. Then, there arise following two questions: whether LRRC8A is essentially involved in the VSOR function in mouse cells as well, and whether LRRC8A represents a missing factor in human VSOR-deficient KCP-4 cells that are derived from the parental VSOR-rich KB cells. Thus, we addressed these questions in the present study. When transfected with siRNA against LRRC8A, the VSOR current was largely reduced not only in human HeLa and KB cells but also in mouse C127 cells. The microarray data analysis showed that the expression level of LRRC8A in KCP-4 cells is not much different from that in KB cells. Also, overexpression of LRRC8A in KCP-4 cells failed to rescue the VSOR activity. These results indicate that LRRC8A is involved in the VSOR function not only in human but also in mouse cells, and loss of VSOR currents in KCP-4 cells is not due to lack or insufficiency of expression of the LRRC8A gene.

(COI: No)

P1-050

β2-adrenoceptor-mediated regulations of the spontaneous rate and ion currents in guinea-pig cardiac cells

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The β1- and β2-adrenoceptors (ARs) are functionally expressed in the heart tissues, with the predominance of the β1ARs subtype. The ratio of β1/β2AR is high in the ventricles and relatively lower in atria and sinoatrial node (SA). Compared to β1AR, β2AR-mediated functional modulation has not been fully elucidated. In the present study, the effect of β2AR stimulation on the spontaneous action potentials, on the L-type Ca^{2+} current ($I_{Ca,L}$) and on the slow component of delayed rectifier K^+ current (I_{Ks}) was examined in guinea-pig cardiac cells. Zinterol in the presence of CGP20712A (β1AR antagonist) and isoproterenol in the presence of ICI118551 (β2AR antagonist) were used for activation of β2 and β1ARs, respectively. In SA cells, similar to β1AR stimulation, β2AR stimulation produced an increase in firing rate of spontaneous excitation. In addition, β2AR stimulation significantly enhanced $I_{Ca,L}$ current and shifted current activation to negative potentials in SA cells. Whereas β2AR stimulation had no effect on $I_{Ca,L}$ in atrial and ventricular cells. Moreover, β2AR stimulation also increased I_{Ks} current in SA and atrial cells, but failed to enhance I_{Ks} in ventricular cells. β2AR stimulation significantly shifted I_{Ks} current activation to negative potentials. These results suggest that in guinea pig heart, β2AR stimulation has a crucial function in the regulation of spontaneous pacemaker activity and main ion currents during sympathetic excitation.

(COI: No)

P1-051

Single channel analysis of the TRPM3 channel in planar lipid bilayers

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TRPM3 is a non-selective cation channel and activated by nifedipine and neurosteroid, pregnenolone sulfate (PS). TRPM3 is expressed in various tissues, including central and peripheral nervous systems, and reported to be involved in heat sensation. However, the functional characterization of TRPM3 channels and its regulation by agonists still remain poorly understood. To investigate the single channel properties of TRPM3, we aimed to incorporate the purified TRPM3 protein in planar lipid bilayers. We investigated the TRPM3 channel activity upon different agonists' activation. Application of nifedipine resulted in dose-dependent increase of open probability of the channel. Unlike nifedipine, application of PS alone did not induce the channel openings and co-application of the PI(4, 5)P_2 or clotrimazole was required. TRPM3 channel currents demonstrated outward rectification upon activation with PS and PI(4, 5)P_2, while linear current-voltage relationship upon activation by PS and clotrimazole. In addition, we found that the channel did not exhibit strong temperature dependence. Increase in temperature up to 42 ℃ did not induce the channel openings in the absence of agonists. While, in the presence of nifedipine, TRPM3 currents demonstrated weak temperature dependence. These results indicate that TRPM3 is unlikely to represent a temperature sensor by itself and some alternative molecular mechanisms may be involved in the temperature sensation.

(COI: No)

P1-052

Expression of ion-channel proteins in the cutaneous mechanoreceptors of mice model of atopic dermatitis

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Atopic dermatitis is chronic dermatitis associated with an extremely itchy and inflamed skin. The patient's skin is commonly very sensitive to external stimuli or irritants, and scratching can increase the severity of dermatitis. Therefore, controlling the itchy skin sensation is important for the treatment of atopic dermatitis. In this study, to clarify the expression of ion-channel proteins that may cause the itchy skin sensation, we immunohistochemically examined cutaneous mechanoreceptors in a mouse model of atopic dermatitis (NC hairless mice). Atopic dermatitis was induced by repeated applications of 2, 4-dinitrofluorobenzene (DNFB) to the ear and back skin of NC hairless mice. Immunohistochemical analysis showed positive reactions for ASIC and TRP proteins in the palisade nerve endings of the auricular skin. These channel proteins may cause the itchy skin sensation that characterizes DNFB-induced atopic dermatitis in NC hairless mice. This study was supported by a Grant-in-Aid for Scientific Research (C) (No. 24590253).

(COI: No)

P1-053

Immunohistochemical distribution of interleukin-18 receptor α in the hypothalamus of male mice

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Interleukin-18 (IL-18) is a pro-inflammatory cytokine and an important mediator of peripheral inflammation and host defense response. IL-18 performs its biological activities through the IL-18 receptor (IL-18R), which consists of two chains, an IL-18-binding α chain (IL-18Rα) and a signaling β chain. IL-18R is widely expressed in the brain, however little is known about the detailed expression of IL-18R in the neurosecretory cells. In the present study, we investigated, by immunohistochemistry, the distribution of IL-18Rα in the hypothalamus of male mice. IL-18Rα-positive cell bodies and fibers were found scattered throughout the organum vasculosum of the laminae terminalis (OVLT) / preoptic area (POA) region. In the median eminence (ME), where the neurosecretory terminals are located, strong IL-18Rα-immunoreactive fibers were also detected. Outside the hypothalamus, IL-18Rα antibodies labeled fibers and cell bodies in the medial septal nucleus (MS), and the nuclei of the vertical and horizontal limbs of the diagonal band (VDB and HDB). In the MS, VDB, HDB, OVLT/POA region, and ME, it has been well known that gonadotropin-releasing hormone (GnRH) neuronal somata and/or fibers were distributed. Therefore, we performed double-label immunofluorescence for IL-18Rα and GnRH. IL-18Rα was expressed on some GnRH-immunopositive cell bodies and nerve fibers. These observations suggest that IL-18 may exert direct effects upon the GnRH neuronal system via IL-18Rα.

(COI: No)

P1-054

Involvement of ion channels in cold allodynia in a new rat model of peripheral arterial disease

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Aim of Investigation: Patients with peripheral arterial disease (PAD) often suffer from peripheral hypersensitivity to cold stimulation which precedes chronic ischemia-induced pain. However, the pathophysiology of cold hypersensitivity has not been fully understood due to the lack of an adequate animal model. In this study, to demonstrate mechanisms underlying ischemia-induced pain associated with PAD, we investigated the role of ion channels on cold allodynia in a newly developed rat model of PAD.
Methods: Under sodium pentobarbital anesthesia, the left common iliac and iliolumbar arteries were ligated respectively, through a midline laparotomy (PAD rats). Sham rats were exposed the arteries without ligation under anesthesia. Von Frey test, plantar test, acetone test were performed, respectively. We also examined alteration of paw withdrawal response to cold temperature. Histological examination of hindpaw skin was performed on day 4 and 10 after the arterial ligation. Effects of selective antagonist to cold-gated ion channels transient receptor potential melastatin 8 (TRPM8), and transient receptor potential ankyrin 1 (TRPA1) channels, were evaluated on behavioral responses to cold stimulus.
Result: Mechanical allodynia was observed for 7 days and cold allodynia was observed from day 7 to 14 after ligation. Intraplantar injection of the antagonists against TRPA1 suppressed cold allodynia in PAD rats.
Conclusions: TRPA1 may play an important role in developing ischemia-induced cold allodynia associated with PAD.

(COI: No)

P1-055

Gene expression analysis of ASIC subtypes in adult-born hippocampal neurons in mice

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Adult hippocampal neural progenitors continuously give rise to dentate granule cells throughout life. Accumulating evidence suggests that adult hippocampal neurogenesis is linked to learning and memory. ASIC1a (acid-sensing ion channel-1a) is a neuronal proton-gated cation channel expressed in mammalian central and peripheral nervous systems. In the hippocampal neurons, ASIC1a is located at the post-synaptic surface of the cells, and receives extracellular protons released from synaptic vesicles. The resultant activation of the channel allows Ca2+ ions to enter into the neurons, contributing to synaptic plasticity and spatial memory in the hippocampus, suggesting that ASIC1a may be directly implicated in the hippocampal neurogenesis. To test this possibility, we are currently investigating the temporal expression of ASIC1a (including other ASIC subtypes) in adult-born hippocampal neurons in mice by single-cell RT-PCR, in situ hybridization, and patch-clamping.

(COI: No)

P1-056

The Gi/o coupled muscarinic receptors form signaling complex with the G protein dependent inwardly rectifying potassium channel

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Excitable cells, such as neurons and muscles, are negatively regulated by the G protein dependent inwardly rectifying potassium (GIRK) channel, which is activated by $G_{\beta\gamma}$ subunits released mostly from Pertussis toxin (PTX) sensitive $G_{\alpha i/o}$ subunits. It has also been reported that the $G_{\beta\gamma}$ subunits released from PTX resistant G_{α} subunits, such as $G_{\alpha s}$ and $G_{\alpha q}$, activate the channel only when the coupled receptors are highly expressed. It is possible that a shorter distance from the channel is the reason of coupling in the case of high surface expression. In this study, we ligated the receptor and the channel by various lengths of linkers of glycine rich amino acid residues, and examined the effects of the linker length on the channel activation. When the number of the linker residues was 100 or less, the Gq coupled muscarinic receptor type 1 (M_1R) evoked the GIRK current upon application of the agonist, whereas the GIRK channels were hardly activated when the number of the linker residues was 268 or more. The FRET efficiency between the M_1R-YFP and the GIRK-CFP was decreased in accordance with the increase in the linker size. In contrast, when the Gi/o coupled muscarinic receptors were used instead of the M_1R, increases in the linker size did not affect the efficiency of the GIRK channel activation and the FRET efficiency. Taken together, the Gi/o, but not Gq, coupled receptors are shown to stay adjacent to the GIRK channel to form the signaling complex.

(COI: No)

P1-057

LC-MS/MS analysis of an orphan metabotropic receptor Prrt3 complex

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Proline rich transmembrane receptor 3 (Prrt3) contains seven transmembrane domains like other metabotropic receptors including mGluRs and $GABA_BRs$. However, the physiological function of Prrt3 and its signaling cascade remain unknown. In our previous study, we revealed Prrt3 expression in mouse brain especially in thalamus and hippocampus, and its possible role in memory retention using Prrt3 heterozygous knock out (KO) mouse. In this study, we identified Prrt3 binding proteins using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Physiologically expressed Prrt3 protein complexes were isolated from wild-type (WT) mouse brain by immunopurification. To eliminate non-specific protein bands, Prrt3 homozygous KO mouse brain sample was used as a negative control. The specific protein bands observed in the silver staining were extracted and analyzed by LC-MS/MS. The protein bands sized between 120 and 220 kDa contained excitatory amino acid transporter 2 (EAAT2), and the band sized 40 kDa contained $G\alpha_o$ protein. The presence of these proteins was further confirmed by western blotting experiment. It is known that dopamine transporter forms a complex with GPCRs (D_2R or GPR37) to regulate the function of dopaminergic synapses. Our finding suggests a possibility that the interaction between Prrt3 and EAAT2 may play an important role in glutamatergic signaling.

(COI: No)

P1-058

Insulin transcriptionally regulated NKCC and CFTR Cl- channel expression through PI3K activation and ERK inactivation in renal epithelial A6 cells

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Insulin is known to stimulate epithelial Na+ channel-mediated Na+ reabsorption in renal epithelial A6 cells, however the insulin action on the Cl- secretion is not fully understood. In this study we investigated the insulin action on Na+-K+-2Cl- cotransporter (NKCC)-mediated Cl- secretion in renal epithelial A6 cells. Insulin treatment significantly enhanced the forskolin-stimulated Cl- secretion with an increase in apical Cl- conductance by upregulating mRNA expression of both NKCC and CFTR Cl- channel. We next examined a role of PI3K on the insulin-induced enhancement of Cl- secretion, because PI3K is a major signal molecule in the insulin cascade. LY294002 (a specific inhibitor of PI3K) decreased Cl- secretion by suppressing NKCC mRNA expression. On the other hand, we found that PD98059 (a MEK inhibitor) further enhanced the insulin-stimulated CFTR mRNA expression and the Cl- secretion in forskolin-treated A6 cells. Furthermore, insulin activated Akt as an indicator of PI3K and inactivated ERK. These observations suggest that insulin enhances forskolin-stimulated Cl- secretion through transcriptional regulation of NKCC and CFTR Cl- channel via PI3K activation and ERK inactivation in renal epithelial A6 cells.

(COI: No)

P1-059 (AP-4)

Cancer cell-specific crosstalk between Na^+, K^+-ATPase and volume-sensitive anion channel in membrane microdomains exerts anti-proliferative activity

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Na^+, K^+-ATPase is a potential target for anti-cancer therapy, because cardiac glycosides, inhibitors of Na^+, K^+-ATPase, potently block cancer cell growth. However, the mechanism underlying the anti-cancer effects of cardiac glycosides is not fully understood. In the present study, we found that ouabain, a cardiac glycoside, inhibited cancer cell proliferation via activation of volume-sensitive outwardly rectifying (VSOR) anion channel. The effects were suppressed by DCPIB, a selective inhibitor of VSOR channel, and the knockdown of Na^+, K^+-ATPase α1-isoform (α1NaK) or VSOR channel component LRRC8A (SWELL1). The disruption of membrane microdomains by methyl-β-cyclodextrin and the attenuation of the production of reactive oxygen species (ROS) by the inhibitors of NADPH oxidase (NOX) significantly suppressed the ouabain-induced VSOR activation and inhibition of cell proliferation. On the other hand, the ouabain-induced effects were not observed in non-cancer cells. These results suggest that α1NaK, NOX and VSOR channels form a signalosome in the membrane microdomains of cancer cells, and that the cardiac glycoside exerts anti-cancer activity through the cancer-specific signalosome.

(COI: No)

P1-060

Redox signal-mediated sensitization of Transient Receptor Potential Melastatin 2 (TRPM2) to temperatures affects insulin secretion from the pancreatic β-cells

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Pancreatic β-cells play a crucial role in blood glucose regulation as they secrete hypoglycemic insulin when blood glucose levels are elevated. In β-cells, reactive oxygen species (ROS) including H_2O_2 are generated in response to many kinds of signals including blood glucose elevation. Interestingly, expression levels of catalase and glutathione reductase, are extremely low in the pancreas compared with other tissues, suggesting that ROS could function as favorable signaling molecules in β-cells.

TRPM2 is a Ca^{2+}-permeable cation channel and expressed in various tissues including brain, spleen and β-cells where TRPM2 is continuously affected by body temperature. We previously found a regulation mechanism of TRPM2 activity whereby its heat-evoked response is dynamically elevated by H_2O_2, termed "sensitization" enabling TRPM2 to be activated by body temperature with temperature threshold reduction.

In WT β-cells, H_2O_2 enhanced heat-evoked $[Ca^{2+}]_i$-increase but the effect was not observed in TRPM2KO cells. Moreover, the N-acetyl cysteine (NAC), an antioxidant compound, -sensitive fraction of insulin secretion by Wt islets was increased with temperature elevation. This temperature-dependent enhancement was not observed in TRPM2KO islets. These data suggest that endogenous redox signals in pancreatic β-cells elevate insulin secretion via TRPM2 sensitization and activity at body temperature.

(COI: No)

P1-061

The regulatory mechanisms of adiponectin in activity of hypothalamic POMC and NPY neurons

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Aim: It has been suggested that serum adiponectin secreted from adipocytes could enter to cerebrospinal fluid and regulate brain functions, in addition to regulation of insulin sensitivity and energy expenditure in the peripheral organs. We have reported that adiponectin facilitates activity of proopiomelanocortin (POMC) neuron, whereas it suppresses activity of neuropeptide Y (NPY) neuron activity in the hypothalamic arcuate nucleus. The aim of this study was to determine the regulatory mechanisms of adiponectin in the activity of NPY and POMC neurons.
Methods: The effects of adiponectin on the membrane potential and electrical firing in POMC neurons in brain slices from POMC-GFP mice were recorded by patch-clamp analysis under current clamp mode. Inhibitory postsynaptic current (IPSC) in NPY neurons of NPY-GFP mice was recorded under voltage clamp mode.
Results: Membrane potential on the ARC POMC neurons was depolarized by bath application of adiponectin, and its depolarization effect was abolished by PI3K inhibitor. IPSC amplitude on NPY neuron was significantly increased by adiponectin treatment.
Conclusion: Adiponectin activates POMC neurons via PI3K pathway and inhibits NPY neurons via facilitation of inhibitory input at postsynapses in ARC. These effects may be implicated in suppression of food intake by adiponectin.
(COI: No)

P1-062

Morphological characterisation of L5 pyramidal neurons of known synaptic properties in rat visual cortex at early developmental age

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The mammalian neocortex comprises neuronal columns that respond to specific sensory stimulus and each column has 6 horizontal cell layers segregated by cell morphology and physiological properties. This well-established laminar organisation suggests that neurons of distinct morphology are most likely to represent discrete functional classes and form specific synaptic connections. This study aims to analyse morphological variables of visual cortex layer 5 (L5) pyramidal neurons of known synaptic properties at early developmental age, and to determine whether these characteristics vary with maturation. Multineuronal whole-cell recordings were made from large and thick-tufted L5 pyramidal neurons in slices from rats aged over the first postnatal month. Altogether 30 sets of Alexa Fluor 488-labelled L5 neurons were included in this study, of which 14 were from P11-P15 rats, one at P20 and the remaining were of age P25-P29. The mean somatic area and somatic perimeter of all 61 neurons were 119.4 ± 25.3 μm^2 and 39.5 ± 4.2 μm, respectively. Intrinsic electrophysiological and synaptic properties of these neurons were shown to develop in parallel after birth. Further investigation is necessary to determine correlation (if any) between morphological maturation and synaptic dynamics of specific neuronal classes in the developing visual cortex.
(COI: No)

P1-063

Role of astrocyte-expressed FABP7 on morphology and synapse Formation of cortical neurons

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Fatty acid binding protein 7 is expressed by astrocytes in the CNS and has been associated with human neuropsychiatric disorders. Here we examined relevance of astrocytic FABP7 in regulation of neuronal dendrite morphology and synapse formation in medial prefrontal cortex (mPFC) by using *Fabp7* KO mice. Golgi staining on mPFC of *Fabp7* KO mice revealed aberrant dendritic morphology and decreased spine density of pyramidal neurons compared with wild-type (WT) littermates. Consistently, primary cortical neurons co-cultured with FABP7-deficient astrocytes exhibited aberrant dendritic morphology, and such change was also observed when neurons were grown in *Fabp7* KO astrocyte conditioned medium. Number of excitatory synapses was decreased in mPFC of *Fabp7* KO mice as well as in neurons co-cultured with *Fabp7* KO astrocytes. Whole cell voltage-clamp recording in brain slices from pyramidal cells of mPFC showed that both amplitude and frequency of action potential-independent miniature excitatory postsynaptic currents (mEPSCs) were decreased in *Fabp7* KO mice. Moreover, hyperactivity of *Fabp7* KO mice in open-field test was partially restored by transplantation of WT astrocytes into mPFC. Collectively, our study suggests that astrocytic FABP7 is important for growth of dendritic arbors and synapse formation of cortical neurons, and further for behavioral control. These findings provide new insights into links between FABP7, lipid homeostasis and neuropsychiatric disorders like schizophrenia.
(COI: No)

P1-064

Ca^{2+} current facilitation underlies short-term facilitation at inhibitory synapses between Purkinje cells

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Cerebellar Purkinje cells (PCs) form synapses not only on deep cerebellar nuclei (DCN) neurons, but also on other PCs through axon collaterals. Opposite forms of short-term plasticity take place at the PC output synapses: while PC-DCN synapses exhibit paired-pulse depression, paired-pulse facilitation (PPF) occurs at the PC-PC synapses (Orduz and Llano, 2007). To clarify a key factor determining those opposite forms of short-term plasticity, we here focused on the mechanism underlying short-term facilitation at PC-PC synapses. As a mechanism for short-term facilitation, the following candidates can be conceived: temporal summation of residual Ca^{2+}, augmentation of action potential (AP) amplitude, saturation of Ca^{2+} buffer protein, facilitated Ca^{2+} influx through voltage-gated Ca^{2+} channels, and Ca^{2+}-dependent positive modulation of vesicle release machinery. Among these, the Ca^{2+} buffer saturation hypothesis was recently shown to be unlikely because of the resistance of PPF to the genetic ablation of two major Ca^{2+} buffering proteins expressed in PCs, calbindin and parvalbumin (Bornschein *et al*, 2013). To test the other possibilities, using dissociated cerebellar cultures we performed fluorescent imaging of residual Ca^{2+} increase in PC axon terminals, and also attempted to directly whole-cell record APs and Ca^{2+} currents from a PC axon terminal. We present the data suggesting that facilitated Ca^{2+} influx into the presynaptic terminal during repetitive stimulation contributes to augmented release probability of synaptic vesicles.
(COI: No)

P1-065

Kinetics of Synaptic Vesicle Refilling with GABA

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Information transfer between neurons requires temporal and spatial balance of excitatory and inhibitory synaptic inputs. After releasing neurotransmitter, synaptic vesicles are retrieved by endocytosis and recycled to be reused for synaptic transmission. To maintain the synaptic efficacy, vesicles must be refilled with neurotransmitter during recycling. Vesicle trafficking and pools have been studied in great detail but much less is known about where and how vesicles are filled with GABA via VGAT. After exocytosis vesicles retrieved by clathrin-mediated endocytosis are rapidly acidified and recycled. Refilling of GABA might be a rate-limiting phase of recycling vesicles. The refilling speed estimated in isolated or reconstructed vesicles seems too slow to fill up vesicles within the period of recycling. Here we re-examined the vesicle refilling rate directly at GABAergic synapses in slices. We made caged GABA photolysis in simultaneous whole-cell recording from a presynaptic inhibitory interneuron and a postsynaptic Purkinje cell in cerebellar cortex. After washing out vesicular GABA in an interneuron with a whole cell pipette containing no GABA, refilling of vesicles with uncaged GABA caused a recovery of IPSCs in a Purkinje neuron within 3 min. Thus, the time course of refilling in situ was significantly faster than that reported in reconstituted or isolated vesicles (5 - 10 min).
(COI: No)

P1-066

Rational design of a novel high-affinity, ultrafast, red calcium indicator R-CaMP2

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Fluorescent Ca^{2+} reporters are widely used as readouts of neuronal activities. Here, we designed R-CaMP2, a novel high-affinity red genetically encoded calcium indicator (GECI) with a Kd for Ca^{2+} < 70 nM, and with a Hill coefficient near 1. Use of the calmodulin-binding sequence of CaMKK-α / β in lieu of a M13 sequence resulted in three fold faster kinetics than R-CaMP1.07 in rise and decay time of Ca^{2+} transients. These features allowed to resolve single action potential (AP) and fast AP trains up to near 20-40 Hz in cortical slices. *In vivo* imaging of the barrel cortex layer 2/3 neurons revealed reliable recording of single APs in R-CaMP2-expressing neurons, while synaptic Ca^{2+} transients were robustly detected in individual dendritic spines with similar efficacy as previously reported ultrasensitive green GECIs. Combining green and red GECIs, we successfully achieved dual-color monitoring of neuronal activities of distinct cell types, in the mouse cortex and in free-moving C. elegans. Together, R/G-CaMP imaging using R-CaMP2 provides a powerful means to interrogate orthogonal and hierarchical active ensembles, thus significantly enhancing our current capacity for functional mapping of neuronal circuits *in vivo*.
(COI: No)

P1-067 (AP-2)

Identification of retrograde signals required for synapse elimination in the developing brain

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Precise formation of neural circuits during development is a prerequisite for proper brain functions. Neurons form exuberant synapses with target cells early in development. Then, necessary synapses are selectively strengthened whereas unnercessary connections are weakened and eventually eliminated during the course of postnatal development. This process is known as synapse elimination. Synapse elimination is an important step to shape initial redundant neural circuits into functionally mature circuits, and the disruption is likely linked to mental disorder and brain dysfunction. While the underlying mechanism is still unclear in any systems, several lines of evidence suggest that retrograde signaling from postsynaptic cells regulates synapse elimination. However, these retrograde signals remain to be identified. We have screened retrograde molecules required for synapse elimination of climbing fiber to Purkinje cell connection in the developing cerebellum. We identified some key retrograde molecules which strengthen necessary synapses and eliminate unnecessary synapses. Here I am going to talk about the role of these retrograde molecules in synapse elimination.

(COI: No)

P1-068

Pharmacological analysis of the inhibitory actions of 5-HT on the excitatory transmission in the dentate granule cells

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The dentate gyrus in hippocampus receives serotonergic innervation from the raphe nuclei, suggesting that synaptic activities in the dentate gyrus are modulated by serotonin (5-HT). Externally applied 5-HT is known to regulate the GABAergic transmission, which indirectly affects the excitatory drive onto the granule cells. However, a possible direct action of 5-HT on the excitatory transmission in the granule cells is not well examined. We previously showed that 5-HT reduces the input resistance (IR) of granule cells, thereby affects the amplitude of EPSPs irrespective of the inputs. We also found that 5-HT specifically reduces the EPSPs evoked by the stimulation of the lateral perforant path (LPP) but not the medial perforant path and mossy cell fibers. In the present study, we examined which types of receptors are involved in the inhibitory modulation of the LPP-granule cell synapse by 5-HT. The reduction of IR by 5-HT was completely blocked by a 5-HT_{1A} antagonist, WAY100635, but the inhibitory effect of 5-HT on the EPSP amplitude was only partially suppressed. By contrast, co-application of a 5-HT_2 antagonist, ritanserin with WAY100635 blocked the inhibitory action completely. These results suggest that the activation of 5-HT_{1A} receptor inhibits excitatory transmissions in the granule cells globally, and that the activation of 5-HT_2 receptor specifically depresses the LPP-granule cell synapse.

(COI: No)

P1-069

Analysis of spontaneous slow currents in inferior olivary neurons

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Inferior olivary neurons (IONs) are the only source of climbing fibers innervating cerebellar Purkinje cells, which have important roles in cerebellar functions. To examine factors to control firing of IONs, we recorded spontaneous currents electrophysiologically. Coronal slices were prepared from C57BL6 mice, and whole cell recordings were performed from IONs. Spontaneous currents with relatively shorter duration were blocked by AMPA and $GABA_A$ receptor blockers (NBQX and bicuculline), suggesting that these were synaptic currents. In addition to the synaptic currents, we found that spontaneous currents with long durations were frequently observed in IONs. These slow currents could be further divided into two subgroups. One group was inward currents which were always followed by an outward current (bipolar currents). The bipolar currents were blocked by TTX, a blocker of the voltage dependent Na^+ channels, or carbenoxolone, a blocker of gap junction, suggesting that these were Na^+ spike from neighboring IONs via gap junctions. The other was inward currents with slow kinetics (slow inward currents). The slow inward currents were TTX resistant and suppressed by bath-applied Ni^{2+}, suggesting contribution of the T-type voltage dependent Ca^{2+} channel (T-type VDCC). To confirm this point further, we used Cav3.1, a major alpha subunit of the T-type VDCC in IONs, null mice. In these mice, the slow inward currents were totally abolished. These results suggest that activation of IONs is regulated by spontaneous generation of these currents.

(COI: No)

P1-070

Input specificity of giant miniature EPSC in hippocampal CA3 neurons

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Miniature EPSCs with exceptionally large amplitude, so-called giant minis, are recorded from hippocampal CA3 neurons. These large events are thought to originate from the mossy fiber synapses, since a lesion of the dentate gyrus decreases giant minis and large clear vesicles exist in the mossy fiber terminals. However, there is no direct evidence for the mossy fiber origin of giant minis. In this study, we examined strontium-induced asynchronous EPSCs (aEPSCs) by the stimulation of different inputs to identify the origin of giant minis. In the solution containing strontium instead of calcium ions, delayed asynchronous release follow evoked-EPSCs composed of synchronous release of multiple vesicles, offering the way to evaluate the input specific size of quantal events. Acute hippocampal slices were obtained from C57BL/6J mice (p13 - 21). Repetitive stimulation (3 pulses at 50 Hz) was applied to the mossy fiber or the associational/commissural (A/C) fiber, and the evoked EPSCs were recorded from CA3 pyramidal neurons. With the stimulation of A/C fiber, amplitudes of aEPSCs observed during 350 ms after the stimulation was not different from those of miniature EPSCs recorded during 350 ms before the stimulation ($p > 0.05$, K-S test, $n = 9$). On the other hand, giant minis (> 80 pA in this study) were observed with the stimulation of mossy fiber ($p < 0.01$, $n = 7$). The time courses of appearance of giant minis matched with aEPSCs observed in other synapses (< 400 ms). These results suggested that giant minis are exclusively originated from the mossy fiber-CA3 synapse.

(COI: No)

P1-071

Involvement of diacylglycerol kinase β in the spine formation at distal dendrites of striatal medium spiny neurons

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Spine formation, a salient feature underlying neuronal plasticity to adapt to a changing environment, is regulated by complex machinery involving membrane signal transduction. The diacylglycerol kinase (DGK) family, which is involved in membrane lipid metabolism, catalyzes the phosphorylation of a lipid second messenger, diacylglycerol (DG). Of the DGKs, DGK β is characterized by predominant expression in a specific brain region: the striatum. We previously demonstrated that DGK β is expressed selectively in medium spiny neurons (MSNs) and that it is highly enriched in the perisynaptic membrane on dendritic spines contacted with excitatory terminals. Moreover, DGK β regulates spinogenesis through actin-based remodeling in an activity-dependent manner. However, the detailed mechanisms of spinogenesis regulation and its functional significance remain unclear. To address these issues, we performed Golgi-Cox staining to examine morphological aspects of MSNs in the striatum of DGK β-knockout (KO) mice. Results show that striatal MSNs of DGK β-KO mice exhibited lower dendritic spine density at distal dendrites than wild-type mice did. We also sought protein targets that interact with DGK β and identified the GluA2 AMPA receptor subunit as a novel DGK β binding partner. In addition, DGK β-deficient brain exhibits significant reduction of TARP γ-8, which represents a transmembrane AMPA receptor regulatory protein. These findings suggest that DGK β regulates the spine formation at distal dendrites of MSNs, presumably through GluA2 receptor-mediated mechanism.

(COI: No)

P1-072

Contextual memory encoding induces a quick change of post-synaptic current in hippocampal CA1 neurons

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The hippocampus plays a central role in contextual learning and memory. Since the learning strengthens both excitatory and inhibitory CA1 synapses, each CA1 neuron shows high diversity of post-synaptic currents (Mitsushima et al., Nature Commun, 2013). In the present study, to examine whether encoding of memory strengthens the CA1 synapses, we examined the temporal change of miniature excitatory and inhibitory post-synaptic currents (mEPSC and mIPSC) in IA-trained rats. As a learning model, we employed inhibitory avoidance (IA) task, and acute brain slices were prepared for patch clamp analysis. Untrained rats showed relatively small mEPSC and mIPSC amplitudes with low diversity of post-synaptic currents. Conversely, IA rats showed higher mEPSC and mIPSC amplitudes from the 5 to 30 min periods after encoding session in IA but not immediately follow this test (at the 0 min). In addition, mIPSC frequency increased from 0 to 5 min periods after encoding session whereas mEPSC frequency increased at the 5 min periods after encoding session, suggesting that memory encoding induced a quick change of mEPSC and mIPSC. Moreover, bath treatment of CNQX (an AMPA receptor antagonist, 10 μM) consistently blocked the mEPSC responses. In contrast, bath treatment of bicuculline methiodide (a $GABA_A$ receptor antagonist, 10 μM) consistently blocked the mIPSC responses. For these results, we conclude that a quick change of AMPA receptors and $GABA_A$ receptors in hippocampal CA1 neurons plays role for encoding of contextual memory.

(COI: No)

P1-073

Simulation analysis of water and ion dynamics in astrocyte

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In response to perturbation of extracellular environment in the brain, astrocyte transport water and ions to maintain proper environment for neural activity. Extracellular K^+ concentration ($[K^+]_{out}$) in the brain increases in response to ischaemia, hypoxia, hypoglycaemia, seizures, and spreading depression and can cause significant problems in brain function. High $[K^+]_{out}$ also induces swelling in astrocytes, leading to cytotoxic edema and cell death in the brain. On the other hand, when the blood brain barrier (BBB) is disrupted, water flux to extracellular and increase of extracellular space lead to vasogenic edema. In order to prevent the harmful elevation of $[K^+]_{out}$ and increase of extracellular space, astrocytes clear excessive K^+ and water in extracellular space by redistributing K^+ and water. Despite the importance of the redistribution function for ion and water, the underlying mechanisms remain unclear. Here we report results from a simulation analysis of water and ion dynamics in astrocyte. The astrocyte models were improved from out previous astrocyte models, which were constructed by incorporating various mechanisms such as intra/extracellular ion concentrations and models of ion channels and transporters. New models of ion channels, such as volume- regulated anion channel (VRAC) were incorporated into the previous model to reproduce regulatory volume decrease, and were used to simulate not only K^+ transport but also water transport under vasogenic edema. Our simulation analysis revealed controversial mechanisms of astrocytic ion and water clearance.

(COI: No)

P1-074

Methionine ameliorates the elevated GAD67 expression in cingulate cortex and the abnormal behaviors of FABP3 KO mice

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Introduction: Fatty acid binding protein (FABP) 3 is strongly expressed in GABAergic inhibitory interneurons in cingulate cortical (CC) which is one of the important brain regions for behavioral coordination. We have so far shown that FABP3 gene ablated mice show the increase of glutamic acid decarboxylase 67 (GAD67) expression in CC and the abnormal cognitive and emotional behaviors. In order to explore the mechanism how FABP3 regulates GAD67 expression, we studied whether methionine (MET) administration, which increases DNA methylation, affects the GAD67 expression in CC of FABP3 gene ablated mice and their abnormal behaviors.
Method: Binding of methyl CpG-binding protein 2 (MeCP2) to specific GAD67 CpG-rich promoter sequence was studied by chromatin immunoprecipitation assay. Mice were treated twice a day for 6 days with MET (5.2 mmol/kg, s.c.). Expression of GAD67 mRNA was examined by qPCR.
Result: In the CC of FABP3 gene ablated mice, binding of MeCP2 to GAD67 promoter was significantly decreased compared with wild-type mice. MET administration restored the elevated GAD67 mRNA expression in the CC of FABP3 KO mice back to wild-type levels, and improved their abnormal behaviors.
Conclusion: These findings suggest that DNA hypomethylation and the associated chromatin remodeling underlie the elevation of GAD67 in CC and the abnormal behaviors of FABP3 gene ablated mice.

(COI: No)

P1-075

The ethanol metabolite acetaldehyde induces the sensation of thirst

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Thirst sensation in hangover has been considered to be due to ethanol-induced diuresis, but heavy-alcohol drinking reduces urine volume. In this study, we hypothesized that ethanol metabolite acetaldehyde is thirst-inducing factor in hangover. Male Wistar rats were used in the present study. Ethanol significantly increased water intake. Coadministration of the aldehyde dehydrogenase inhibitor cyanamide with ethanol increased both water and salt intake further and earlier. Urine volume was decreased by ethanol. Acetaldehyde with cyanamide induced water and salt intake. The elicited water and salt intake was suppressed by intraperitoneal and intracerebroventricular injections of angiotensin AT1R antagonist candesartan. c-Fos expression in the circumventricular organs and supraoptic nucleus was increased by acetaldehyde, and the increment was suppressed by central AT1R blockade. Acetaldehyde suppressed blood pressure and increased plasma renin activity. Meanwhile, intracerebroventricular acetaldehyde increased only water intake. In multi-array extracellular recordings from slice preparations of subfornical organ, acetaldehyde showed a direct effect on neuronal cell bodies. Taken together, acetaldehyde may induce thirst sensation via two distinct and previously unsuspected processes, an indirect action through the renin-angiotensin system by a depressor response and a direct action in the dipsogenic centers in hangover, independent of diuresis.

(COI: No)

P1-076

FILIP-related molecule binds to NMDA receptor and controls spine maturation and synaptic function in the hippocampal neuron

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Dendritic spines are small actin-rich structures and the primary post-synaptic sites of excitatory neurotransmission in the brain. The actin cytoskeleton is essential for spine maturation as well as for synaptic plasticity and memory formation. Non-muscle myosin IIb plays a major role for regulation of actin dynamics in the dendritic spines. However, how myosin IIb directly alters cytoskeletal dynamics through ATPase-driven contraction of actin networks and how myosin IIb function is regulated during the dendrite spine maturation are still poorly understood. We found that one FILIP (Filamin A-Interacting Protein)-related molecule, FRM, was a binding partner of myosin IIb and was expressed in the hippocampal and neocortical neurons. When endogenous FRM was knocked down in cultured hippocampal neurons, it inhibited spine shortening for spine maturation and changed the ratio of NMDA receptor expressions on spines. Additionally, we found that FRM was interacted with NMDA receptor. These data suggest that FRM is a new myosin IIb modulator that controls spine maturation and synaptic function in the hippocampus as well as in the cerebral cortex.

(COI: No)

P1-077

Kinetic Organization of Ca^{2+} Signals that Regulate Synaptic Release Efficacy

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Calcium regulation of neurotransmitter release is essential for maintenance of synaptic transmission. However, the temporal and spatial organization of Ca^{2+} dynamics that regulate synaptic vesicle (SV) release efficacy in sympathetic neurons is poorly understood. We investigate the N-type Ca^{2+} channels-mediated kinetic structure of Ca^{2+} regulation of cholinergic transmission of sympathetic neurons. We measured the effect of Ca^{2+} chelation with fast and slow buffers on exocytosis, synaptic depression, and recovery of the readily releasable vesicle pool (RRP), after both single action potential (AP) and repetitive APs. Postsynaptic potentials peaking at ~12 ms after the AP was inhibited by both rapid and slow Ca^{2+} buffers, suggesting that, in addition to the well-known fast Ca^{2+} signals at the active zone (AZ), slow Ca^{2+} + signals at the peak of Ca^{2+} entry also contribute to paired-pulse or repetitive APs responses. Following single AP, discrete Ca^{2+}-transient regulated synaptic depression in a rapid (<30 ms) and slow (<120 ms) phase. In contrast, following prolonged APs trains, synaptic depression was reduced by a slow Ca^{2+} signal regulation lasting >200 ms. Finally, after an AP burst, recovery of the RRP was mediated by an AP-dependent rapid Ca^{2+} signal, and the expansion of releasable SV number by an AP firing activity-dependent slow Ca^{2+} signal. These data indicate that local Ca^{2+} signals operating near Ca^{2+} sources in the AZ are organized into discrete fast and slow temporal phases that remodel exocytosis and short-term plasticity to ensure long-term stability in acetylcholine release efficacy.

(COI: No)

P1-078

Effects of astaxanthin on axonal transport in cultured mouse dorsal root ganglion neurons

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Astaxanthin, a non-provitamin A carotenoid, which is found in the pigment of microalgae, crustacean, salmon and so on. It is well known as an antioxidant, and it has also been reported to have anti-inflammatory and anticancer effects. We have previously demonstrated that astaxanthin suppressed against oxidative stress-induced neurite growth inhibitory. However the direct effects of AX on neuronal functions have rarely examined. Axonal transport is critical for neurogenesis and maintenance of neuronal functions. This study we investigated the effects of AX on axonal transport in cultured dorsal root ganglion neurons. Movement of organelles in neurites was observed by real-time video-enhanced microscopy. Axonal transport in anterograde and retrograde directions had not significantly changed after treatment with astaxanthin. The average velocity of particle movement and the diameter of these neurites were also not significantly changed in both directions.

(COI: No)

P1-079

Hydrogen peroxide-mediated modulation of synaptic transmission in rat spinal ventral horn neurons

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Hydrogen peroxide (H2O2) is produced at high concentrations under pathological conditions. In this study, we examined the presynaptic effects of H2O2 on the rat spinal ventral horn neurons using whole-cell patch-clamp recordings from spinal cord slices. H2O2 (1 mM, 5 min) induced biphasic changes in the frequency of miniature excitatory postsynaptic currents (mEPSC): i.e., initial augmentation and subsequent depression, lasting after 10 min washout. Subsequent depression was attenuated by GABA-A receptor antagonist, indicating that the depression is mediated by the activation of presynaptic GABA-A receptors. Actually, the frequency of GABAergic miniature inhibitory synaptic currents (mIPSC) were increased by superfusing H2O2. This action might protect neurons from an excessive excitation mediated by H2O2. Another H2O2 effect on the mEPSC frequency, initial augmentation, was suppressed by superfusing Ca2+-free solution. Furthermore, N-type voltage dependent calcium channel (VDCC) blocker inhibited completely the initial augmentation and P/Q-type blocker inhibited partially, whereas R- and L-type blockers had no effect, indicating that the augmentation is in part mediated by Ca2+ influx through the N- and P/Q-type VDCC. Meanwhile, the increase of GABAergic mIPSC frequency by H2O2 was not attenuated by Ca2+-free solution. These results suggest that the presynaptic N- and P/Q-type VDCC might represent a novel target for preventing an excessive excitation, which does not attenuate neuroprotective mechanisms such as GABA release.

(COI: No)

P1-080

Effect of long-term STN-HFS on the IPSC of Substantia Nigra pars Reticulata (SNr) Neurons in the slices from reserpinized rat

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I reported that GABAergic IPSC-LTP was induced by high frequency electrical stimulation onto subthalamic nucleus (STN-HFS) through the postsynaptic mechanism in the half of SNr neurons tested (9 out of 17 neurons) under control condition. The pared pulse ratios of 1.692 ± 0.178 before STN-HFS and 1.349 ± 0.068 at after 120 min STN-HFS were not significantly different ($p = 0.103$, $n = 14$). This IPSC-LTP induced by STN-HFS was observed in almost all neurons tested (11 out of 13 neurons) in the solution with 3 or 5 μM Sulpiride, a D_2 dopamine receptor antagonist. The normalized amplitude was 1.878 ± 0.229 at 120 min after STN-HFS. This value was significantly different from the one before STN-HFS ($n = 11$, $p = 0.004$). On the other hand, STN-HFS induced the LTD-like decrease in the amplitude of glutamatergic EPSC at SNr neurons evoked by electrical stimulation onto internal capsule in the solution with 20 μM bicuculline. As reported, this LTD-like effect on EPSC was abolished by D_1 receptor antagonist (5 μM SCH23390). Since a long time patch-recording itself might affect the amplitude of IPSC, I recorded the IPSC amplitude under voltage-clamp for 122 min without STN-HFS in some neurons ($n = 4$). As expected, an IPSC decreased in its amplitude rather than increase. Normalized amplitude was 0.856 ± 0.09. However, in the slices from reserpinized rats, an acute model rat of Parkinson's diseases, STN-HFS did not induced IPSC-LTP in 4 neurons tested. At 90 min after STN-HFS, the normalized amplitude of IPSC was 0.686 ± 0.112. This value was not significantly different from control ($p = 0.107$).

(COI: No)

P1-081

Inhibition of spontaneous GABAergic currents after increasing pre- and post-synaptic activity in neonatal rat hippocampus

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The activity-dependent plasticity of excitatory synapses is considered to be a model of learning and memory. $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs) regulate the excitatory synaptic transmission by modifying activity of the principal cells, but plasticity of the inhibitory synapses has not been sufficiently characterized. Several investigators reported that repetitive depolarization of principal cells facilitates or suppresses inhibitory synaptic transmission. Thus, direction (facilitation or suppression) and mechanism of the plasticity, remain to be established. In the present experiments, $GABA_A$ergic sIPSCs were recorded in acute slices of neonatal rat hippocampus. Using whole cell patch-clamp recording method, effect of the repetitive postsynaptic depolarization on the sIPSCs was determined. Depolarization of postsynaptic neurons alone did not cause marked alteration of the frequency or amplitude of sIPSCs. Simultaneous activation of presynaptic and postsynaptic neurons however, induced transient decrement of the frequency of the sIPSCs. In the presence of antagonists of metabotropic glutamate receptors or an antagonist of CB1 receptor, the inhibition caused by this simultaneous stimulation was suppressed. These results suggest that postsynaptic depolarization and facilitation of glutamate release from presynaptic terminal transiently inhibit the sIPSCs and that CB1 receptor probably participates in this inhibition.

(COI: No)

P1-082

Synapse-specific effects of interleukin-1β on synaptic plasticity in the mouse hippocampus

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Interleukin-1β (IL-1β), which is a key molecule in the inflammatory responses during infection and injury, exerts local effects on hippocampal synaptic plasticity via IL-1 receptors that are present at high levels, especially in the hippocampus. To examine the effects of IL-1β on synaptic plasticity in different hippocampal regions, we examined long-term potentiation (LTP) in acute hippocampal slices obtained from mice, which is considered as the cellular model for learning and memory. IL-1β (1 ng/ml) was applied for 30 min before high-frequency stimulation (HFS: 100 Hz for 1 sec × 3) to induce LTP. LTP was significantly impaired by IL-1β application at the Schaffer collateral-CA1 synapses (138.4 ± 6.2 % vs. 119.1 ± 3.8 %, % of excitatory postsynaptic potential (EPSP) amplitude 60 min after HFS against baselines, mean ± SEM, $n = 4$ respectively, $p < 0.05$, t-test), and at the associational/commissural (A/C) fiber-CA3 synapses (160.9 ± 7.4 % vs. 134.3 ± 9.1 %, $n = 6$, respectively, $p < 0.05$), which are both dependent on NMDA receptor activation. However, mossy fiber-CA3 LTP, which is independent of NMDA receptor activation and expressed presynaptically, was not impaired by IL-1β (155.0 ± 15.6 % vs. 161.2 ± 19.8 %, % of EPSP amplitude 30 min after HFS against baselines, $n = 8$ respectively, $p > 0.05$). Our results show different effects of IL-1β on the LTPs at different kinds of synapses, indicating that IL-1β has synapse-specific effects on hippocampal synaptic plasticity.

(COI: No)

P1-083

Physiological role of N-glycosylation in AMPA receptor-mediated synaptic transmission

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The intracellular molecular mechanisms underlying the regulation of the AMPA receptor have been dramatically elucidated in the past few decades. In contrast, the regulation of the extracellular domain remains unclear. Here, we focused on N-glycosylation of the AMPA receptor in the extracellular domain and tried to clarify their functions by combining molecular biological and electrophysiological techniques. In the last meeting, we presented that the digestion of N-glycosylation of primary hippocampal cultured neurons and/or GluA1 expressing HEK293 cells by a treatment with PNGase-F changed AMPA currents from desensitization to re-sensitization, and that asparagine residues, positioned at 401 of 406, putative N-glycosylation sites, were critical sites for the expression of re-sensitization. In this meeting, we will report a physiological role of N-glycosylation in AMPA receptor-mediated synaptic transmission. First, we examined whether excitatory post-synaptic currents (EPSCs) induce the re-sensitization by treatment of acute brain slices with PNGase-F. Under the whole-cell recordings, single electrical stimulation of Schaffer collateral did not show the re-sensitization in hippocampal pyramidal neurons, however, paired pulse stimulation generated a similar re-sensitization. A mEPSC analysis revealed that PNGase-F treatment exhibited significantly longer the decay time. These results suggested that N-glycosylation modulate the synaptic transmission by altering EPSCs.

(COI: No)

P1-084

Synaptic distribution on single labeled mitral cell in the olfactory bulb

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Mitral cells are major projection neurons of olfactory bulb (OB). They receive olfactory inputs, regulate information, and send their axons to the olfactory cortex. In this study, to understand output control from the OB, we established a method for visualization of single mitral cell and examined quantitative distribution of synapses with their target neurons. Single mitral cell fluoro-labeled by virus injection was then processed for serial-sectioning electron microscopy (EM). EM-reconstructed mitral cell was obtained by approximately 300 serial thin sections each, and synapse distributions and their target neurons were analyzed. Total number of synapse on the cell body was 511: number of output and input synapse was 290 and 221, respectively. Among them, 58 % of output synapses and 76 % of input synapses made reciprocal pairs. These synapses were made by individual 261 profiles and 129 of them involved reciprocal pairs. These EM-findings for synapse on the single mitral cell have been confirmed by multiple fluoro-labeling immunocytochemistry. That is, synaptic and neuronal markers, such as parvalbumin, vesicular gamma-aminobutyric acid transporter, vesicular glutamate transporter, were expected for wider view of synaptic distributions. Then we further confirmed that light-microscopically (LM) identified sites were involved in EM-identified synapses. In conclusion, we demonstrate synapse distribution on both soma and dendrites of the single mitral cell by correlative LM and serial-EM studies.

(COI: No)

P1-085

Structural basis for cholinergic regulation in the mouse olfactory bulb

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Odor information is regulated by olfactory inputs, bulbar interneurons, and centrifugal inputs from other brain regions. Among them, centrifugal inputs have been less analyzed. Cholinergic (ACh) neurons derived from the nucleus of horizontal limb of diagonal band (HDB) are one of the major centrifugal inputs to the olfactory bulb (OB). However, little is known about how ACh neurons make synaptic connections with various bulbar neurons to regulate odor signals. In this study, we focus on ACh regulation of the OB, and analyzed the detailed distribution of ACh neurons in the HDB, and the synapses formation in the OB.

A retrograde tracer, Fluoro-Gold was stereotaxically injected into the OB, and serial slices immunostained with multiple neuronal markers to analyze cellular distribution. To clarify projection pathway of ACh neuron, the HDB neurons were fluoro-labeled by viral injection. We confirmed that the infected neuron was Ach, and then single neuron was traced three-dimensionally. Furthermore, to identify target neurons of ACh fibers in the OB, we performed fluorolabelling with various interneuron markers and observed synaptic formation of them by electron microscopy (EM).

In comparison with the other neuron, ACh neurons located rather medially in the HDB. Fluorolabelling revealed that ACh fibers were associated with bulbar interneurons throughout all layers. EM study showed that ACh fibers made asymmetrical synapses, although their post-synaptic density exhibited variable feature.

Our present study suggests that ACh neurons contribute to elaborate mechanism of olfactory processing in the OB.

(COI: No)

P1-086

Spike timing-dependent plasticity (STDP) at L2/3 intercolumner connections and its interaction with STDP at L4-L2/3 connections

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Deprivation-induced map plasticity requires standard STDP with long-term potentiation (LTP) and long-term depression (LTD) at L4-L2/3 synapses, but for spared columns to drive deprived columns effectively, horizontal connections at L2/3 between adjacent columns might also exhibit some form of plasticity during critical period. We tested this possibility and found that these connections exhibit STDP with LTP only, or both pre-before-post as well as post-before-pre timing produced potentiation in a timing-dependent manner, that is, shorter the timing differences, the larger the potentiation. To have LTP-STDP should be advantageous for the formation of horizontal connections. In addition, we found that the formation of this horizontal intercolumner connection could be facilitated by existing vertical connections between L4-L2/3 that exhibit standard STDP with LTP and LTD, just like an interaction between L4-L2/3 LTP-STDP and thalamus-L2/3 LTD-STDP during the 2nd postnatal week, as we report previously. We conclude that intercolumner STDP between L2/3 would contribute to map plasticity, and that network formation by interaction of STDP might be an important rule for shaping the neural network.

(COI: No)

P1-087

Synaptic potentiation in the central amygdala in trigeminal inflammatory pain model of rats

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Capsular part of the central amygdala (CeC), known as the "nociceptive amygdala", receives direct nociceptive inputs by way of the spino- (trigemino-) parabrachio-amygdaloid pathway (Bernard et al., 1989). The excitatory synaptic transmission from the lateral parabrachial nucleus (LPB) to the CeC neurons (LPB-CeC synapse) shows robust potentiation in various types of pain models in rodents (Veinante et al., 2013). However, such LPB-CeC synaptic potentiation has been demonstrated only in spinal pain models. We examined whether this LPB-CeC transmission is also affected in the models with trigeminal pain. We observed a marked LPB-CeC potentiation as recorded with whole-cell patch clamp in acute brain slices prepared at 6 h after upper-lip injection of 5% formalin in Wistar rats. In a similar manner to the spinal pain models, the LPB-CeC transmission in the right, but not in the left, amygdala was markedly potentiated in a manner being accompanied by decreased paired-pulse ratio, limited relation with the firing pattern of neurons and no apparent changes in NMDA/AMPA ratio. As the neurons in the spinal nucleus of the trigeminal nerve mostly project to the bilateral CeC, these results, which are the first to describe the synaptic potentiation in the trigemino-parabrachio-amygdaloid pathway, support a lateralized nature of the inflammation-induced synaptic potentiation. Supported by Kakenhi.

(COI: No)

P1-088

The formation of climbing fiber synapses with cerebellar Purkinje cell in hypogranular mice

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Cerebellar Purkinje cells (PCs) receive two kinds of excitatory input, numerous parallel fibers (PFs) and single climbing fiber (CF). While numerous PFs run in a vertical direction to sagittal plane, where PC dendrites extend, and form single or a few synapses with each PC dendrite, single CF climbs up along PC dendrite in sagittal plane and forms several hundred synapses exclusively with each PC dendrite. To evaluate the contribution of the PFs and PF synapses upon the formation of CF synapses, we observed developmental changes of CF synapses on PC dendrites under reduced PFs caused by MAM treatment from the set of serial electron-microscopic ultrathin sections including the basal of PC somata and dendritic tips. In hypogranular mice, the arbors of PC dendrites were slightly poor and shortened at adult. Moreover, PCs were innervated by multiple CFs from morphological observation. The multiple innervations are classified into two types, the distal type and the proximal type. The distal type is found in distal PC dendritic portion, i.e., spiny branchlet, whose spines were innervated by adjacent CFs. Such type was found from P12 and the number of the ectopic synapses progressively increased. The proximal type is found in proximal PC dendritic portion, which was dually innervated by associated CF and adjacent CF, or whose dendrite branch was fully governed by adjacent CF. The proximal type was found at P15 the number is not changed. Thus, the mechanism, by which multiple innervation occurs, was different between the distal type and proximal type.

(COI: No)

P1-089

SBF-SEM 3D reconstitution analysis reveals alterations in composition and morphology of mouse hippocampal mossy fiber synapses by afadin knockout

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Afadin plays roles in the formation of puncta adherentia junctions and differentiation of presynapses in hippocampal neurons. Still, little is known about the regulation of synapse composition and morphology by afadin. To elucidate this question, we performed serial block face-scanning electron microscopy (SBF-SEM) and 3D reconstitution on mouse hippocampal mossy fiber-CA3 pyramidal cell synapses (MF synapses) in an afadin-knockout background. We used hippocampal MF synapses because of their large size and distinct structure in which a mossy fiber bouton wraps postsynaptic dendritic spines. We show that puncta adherentia junctions and post synaptic densities are reduced and deformed, and that the coverage ratio of dendritic spines by a mossy fiber bouton is reduced. Moreover, the number of synaptic vesicles of the readily releasable pool is reduced by afadin-knockout implicating impaired neurotransmission. These results indicate that afadin is essential for proper composition and morphology of hippocampal MF synapses.

(COI: No)

P1-090

PACAP is involved in hippocampal neurogenesis after global ischemia

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Pituitary adenylate cyclase-activating polypepetide (PACAP) has been shown to protect neurons during CNS diseases and contributes to neurogenesis during development. However, the role of PACAP on neurogenesis during ischemia has not been understood. C57BL/6 wild-type (WT) and PACAP (+/-) mice were subjected to 12 min transient common carotid artery occlusion (tCCAO). After tCCAO, the mice were administered vehicle or PACAP38 (1 pmol) into the hippocampal dentate gyrus. Another set of mice were injected BrdU (300mg/kg, ip) before sacrifice at days 1, 3, 7, 14, 28 and 56 after tCCAO. Brains were collected followed by 4% paraformaldehyde-fixation and 15 μm sections were prepared for multiple-staining of BrdU and cell markers. The hippocampus injected with vehicle or PACAP38 were immunoblotted for collapsin response mediator protein-2 (CRMP2) to estimate axonal extension. BrdU (+) cells were observed in the subgranular zone of the dentate gyrus and increased after tCCAO. Number of BrdU (+) cells peaked at day 7 and were significantly greater in WT mice compared to PACAP (+/-) mice. BrdU (+) cells were co-labelled with nestin positive neuronal stem cells, but less in S100 and Iba-1 positive glial cells. CRMP2 signal was greater in PACAP treated mice. These results suggested PACAP contributes to neurogenesis in addition to neuroprotection during ischemia.

(COI: No)

P1-091

Oscillatory network formation and cholinergic/histaminergic activity in the cultured olfactory neurons in the slug

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Synchronous oscillatory activity in a laminar structure is common in the olfactory system of both vertebrates and invertebrates. In the terrestrial slugs, periodic oscillation is recorded from the surface of the laminar structure of procerebrum (PC) and its frequency changes are suggested to encode the olfactory information and memory. Acetylcholine and histamine are known to increase the oscillatory frequency in the PC, and is one of the candidates of the neurotransmitters that are involved in such higher cognitive functions. We recently found that oscillatory neuronal network was formed from dispersed cell culture of PC neurons. In the present study, we thus examined whether cholinergic and histaminergic system are present in cultured PC neuronal network or not. First, increases in neurite arborization, neurite connection and cell aggregation were observed with time in culture. Second, in calcium imaging for each PC neurons, acetylcholinesterase inhibitor or nicotine increased the number of calcium transients and induced synchronous oscillatory activity. These results suggest that acetylcholine can function as an excitatory transmitter in cultured PC neuron network via mainly nicotinic acetylcholine receptors activation. Third, histamine increased the number of calcium transients without synchronous oscillatory activity in a smaller number of PC neurons. It suggests the presence of histaminergic receptors in the cultured olfactory neuron network.

(COI: No)

P1-092

Dynamic changes of ACF7 localization during neuronal development

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Coordination between two major cytoskeletal components, microtubules (MTs) and filamentous actin (F-actin), has been shown to underlie diverse cellular functions. ACF7/MACF1 is a mammalian spectraplakin able to bind both MTs and F-actin directly. ACF7 localizes at the tip of growing MTs and coordinates MT dynamics with F-actin in fibroblasts, and is essential for proper neuronal migration and axonal projection in developing mouse brain. Interestingly, previous reports identified ACF7 as a candidate postsynaptic component by proteomic analyses of biochemically purified PSD fractions. These results indicate that ACF7 changes its localization from tip of MTs to postsynaptic sites during neuronal development.

To test this hypothesis, we performed live-cell imaging of GFP-tagged ACF7 in hippocampal neurons. In immature neurons with growing neurites, ACF7-GFP localized at the tip of MTs and showed translocation driven by MT assembly throughout the cytoplasm. However, mature neurons showed few events of ACF7-GFP translocation at the tips of MTs. Time-lapse imaging of ACF7-GFP revealed initial clustering of ACF7-GFP within dendritc shafts and subsequent translocation into dendritic spines. These results suggest that the existence of a molecular switch that converts ACF7 localization during neuronal development. ACF7 may facilitate interaction of MTs penetrating into spines with actin meshwork, and contributes to excitatory synapse development.

(COI: No)

P1-093

Investigation of the cellular structures inside neuronal compartments by two-photon fluorescent correlation spectroscopy

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Mobility of macromolecules within cells is affected by the presence of membranes, cytoskeletal polymers and nuclear chromatin. Therefore, the architecture of intracellular components in neurons, such as dendritic spines and nuclei, is a key factor for molecular interactions and is the basis for neuronal function. Morphological changes of spines during synaptic plasticity are associated with multiple cellular events that regulate the actin cytoskeleton, membranes and postsynaptic density. Similar to spines, the shape of the nucleus changes in response to neuronal activity. This morphological change affects propagation of calcium signal and transcriptional events, which may lead to intra-nuclear heterogeneity of chromatin structure. Precise measurements of molecular dynamics within specific compartments are important, but currently available techniques are not ideal for measurements within neurons with complex three-dimensional morphology. To directly obtain quantitative parameters on molecular dynamics, we are currently testing the possibility of applying two-photon fluorescence correlation spectroscopy (2P-FCS) to monitor dynamics of either biologically inert fluorescence molecules or GFP tagged proteins within spines and nuclei of cultured neurons. At the meeting, we will discuss the relationship between analytical results of 2P-FCS and molecular architecture inside spines and nuclei including organization of the actin cytoskeleton and chromatin structure.

(COI: No)

P1-094

In vivo recordings of optogenetically evoked striatum firings

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We are investigating a firing prolongation of the adult rat striatal neuron after repetitive optogenetic stimulation observed in an acute striatal slice. In this report, we tested whether the prolongation could be observed in vivo. Wistar Thy-1.2 promoter ChannelRhodopsin-2 Venus Rats were anesthetized by urethane and placed in a stereotaxic frame. A wire tetrode was coupled with a 500 μm plastic optic fiber and inserted into the striatum of the rat. We were able to observe the firing prolongation by the tetrode after repetitive LED photostimulation through the optical fiber. The striatal neurons showed a prolonged firing response of gradually increasing duration when exposed to 5 repetitive optogenetic photostimulations. This result indicates that both acute slice and in vivo striatal neurons hold their internal state in decasecond-order timescale.

(COI: No)

P1-095

Mapping of neuronal network activity in dorsal horn during the activation of low threshold afferent fibers: An application of multi-channel array system to acute slices of mouse spinal cord

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Peripheral nerve injury-induced hyperalgesia and allodynia is suggested to be associated with synaptic rearrangement of neuronal circuits in the spinal dorsal horn. However, further details of this synaptic reorganization remain to be elucidated. The present experiments used a multi-electrode array (MEA) system to analyze neuronal circuitry activity in the dorsal horn of the acutely prepared spinal slice preparation. Lumbar spinal cord slices with a dorsal root attached were prepared from an adult mouse anesthetized with ketamine and xylazine. The dorsal root was stimulated with a suction electrode and simultaneous recordings of extracellular field potential were made from 64 points on a spinal slice. Off-line analysis of the amplitude and propagation of evoked field potential (FP) was conducted. Isopotential contour maps of evoked FP were also composed. Stimulation of dorsal root at stimulus intensities of 4-5 times the lowest threshold of afferent fibers evoked negative FP. This negative FP distributed from the superficial dorsal horn into the deep dorsal horn. The amplitude of this negative FP was largest in the laminae III-IV. The negative FP recorded in the lamina II exhibited smaller amplitude and longer latency compared to that recorded in the laminae III-IV. We are currently conducting experiments in mice with partial ligation of the sciatic nerve to show differences in the evoked FP between neuropathic and control mice.

(COI: No)

P1-096

Action potential firing activates myosin II and VI in distinct dynamin isoforms-mediated synaptic vesicle recycling pathways

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Myosin II regulates presynaptic actin dynamics and VI mediates postsynaptic receptors endocytosis in the brain. We have first demonstrated role of myosin II in regulation of transmitter release, however, function of presynaptic myosin VI was not explored. Myosin IIB and VI are expressed at presynaptic terminals superior cervical ganglion (SCG) neurons. Thus, we examined synaptic vesicle (SV) trafficking and the molecular mechanism linking variation in neural activity to SV resupply. Combined genetic knockdown and direct physiological measurement of synaptic transmission from paired SCG neurons in culture show that myosin IIB and VI together cover physiological range of AP firing patterns, mediating replenishment of a shared readily releasable pool (RRP) following distinct endocytic pathways activated selectively by dynamin isoforms. Myosin VI resupplied the RRP with slow kinetics independently of firing rates but acted quickly within 50 ms after AP. Under high frequency AP firing, myosin IIB resupplied the RRP with fast kinetics in a slower time window of 200 ms. Myosin IIB mediates SV resupply from a reserved pool to the RRP in a SV recycling pathway activated by dynamin 1, while myosin VI mediates SV resupply through another pathway activated by dynamin 3. Collectively, our findings show that myosin IIB and VI work individually in distinct vesicle reuse pathways activated by dynamin isoforms, having distinct rate and time constants with physiological action potential frequency.

(COI: No)

P1-097

Analyses of bone cancer pain-related molecules in the spinal cord and the effects of irradiation on the cancer

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Bone cancer pain is a serious problem for patients. To investigate the mechanisms of this pain, we examined the related molecules and the effects of irradiation. To create cancer pain model mice, we injected osteolytic sarcoma cells into their femurs. To assess the pain levels, we used the von Frey test. We compared the expression of glial markers and a mediator of glial activation in the spinal cord between the control and the cancer pain model because glial cells contribute to cancer pain. The protein expressions of the astrocyte marker GFAP, the microglia marker Ibal and the mediator TLR-4 increased in the ipsilateral side in the cancer pain model. A decrease in the K^+-Cl^- cotransporter KCC2 and an increase in the Na^+-K^+-Cl^- cotransporter NKCC1 increased intracellular Cl^- and cause a positive shift in the reversal potential of the $GABA_A$ receptor-induced current. That process contributes to GABA-induced pain facilitation. Real-time RT-PCR analysis showed that the cancer pain decreased KCC2 and increased NKCC1 in the ipsilateral side, and that irradiation increased KCC2 and decreased NKCC1. The gramicidin perforated patch-clamp technique was used to observe the reversal potential shift. The number of neurons which induced the positive shifts in the cancer pain model was higher than that in the control. The trend of the reversal potential shifts in the cancer pain model which received irradiation was similar to that in the control. Our results may provide a clue to elucidate the mechanism that underlies bone cancer pain.

(COI: No)

P1-098

The recycling pool size estimated by different stimulation frequencies at the calyx of Held presynaptic terminal

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For sustained synaptic transmission, it is essential to maintain the recycling pool of vesicles. After exocytosis of synaptic vesicles, fused vesicle membranes are re-internalized by endocytosis, refilled with neurotransmitter and recycled for reuse, but the mechanism regulating this pool size is unclear. In hippocampal autaptic culture, Ikeda & Bekkers (2009) estimated the number of synaptic vesicles per bouton using the vacuolar ATPase blocker bafilobycin A1 (Baf A1) to block vesicle refilling. Their estimates ranged between 100 - 200 vesicles and were independent of stimulation frequency. We asked whether the recycling pool size is regulated by presynaptic activity. By blocking vesicle refilling through either bath-application of BafA1 or washout of glutamate, we found both the amplitude of evoked EPSCs (eEPSCs), and the frequency of miniature EPSCs (mEPSCs) gradually reduced. The reduction rate of eEPSCs comprised both time- and use-dependent components, the former likely caused by a passive leakage of glutamate from vesicles, and the latter by recycling of non-refilled vesicles. The recycling pool size, estimated by dividing the time integral of eEPSCs by that of a mEPSC, was approximately 500/AZ at 20 Hz stimulations, but 10 to 20 times smaller at 0.02 Hz. Neither BafA1 nor glutamate washout affected the change in presynaptic membrane capacitance following stimulation. We cautiously speculate that neuronal activity can regulate the pool size of recycling vesicles.

(COI: No)

P1-099

Role of neuronal Ca^{2+} sensor-1 in learning and memory in mice

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Intracellular Ca^{2+} plays key roles in regulating various functions in the nervous system. Neuronal calcium sensor-1 (NCS-1) is a Ca^{2+} binding protein, which mediates Ca^{2+} signals in a spacial and temporal manner. Although NCS-1-deficient *C. elegans* shows memory dysfunction, the brain functions in NCS-1 knockout (KO) mice have not been examined. Here we investigate whether NCS-1 regulates brain structure, neural functions, and physical activity. Histological analysis revealed that NCS-1 was expressed throughout the brain, but at the highest in hippocampal neurons, a memory center. Morris water maze analysis demonstrated that KO mice had lower functions of spatial learning and memory. Physical activity was not different between WT and KO mice. To understand NCS-1-mediated signaling pathway, we measured the amount of neurotrophic factors in WT and KO groups. In KO mice, BDNF, a key regulator for memory function, was significantly lower in the entire brain, although GDNF and NGF levels were not different from WT mice. Additionally, dopamine secretion was decreased in KO brain. Electron microscopy revealed that the number of large dense core vesicles, which release BDNF and dopamine, was decreased in KO group. These results suggest that NCS-1 plays an important role in spatial learning and memory through the regulation of BDNF and dopamine secretion in the brain.

(COI: No)

P1-100

Upregulation of HP1γ expression during neuronal maturation promotes axonal and dendritic development in mouse embryonic neocortex

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Immature neurons undergo morphological and physiological changes including axonal and dendritic development in order to establish neuronal networks. Since the transcriptional status changes at a large number of genes during neuronal maturation, global changes in chromatin modifiers may take place in this process. We now show that the amount of heterochromatin protein 1 γ (HP1γ) increases during neuronal maturation in the mouse neocortex. Knockdown of HP1γ suppressed axonal and dendritic development in mouse embryonic neocortical neurons in culture, and either knockdown or knockout of HP1γ impaired the projection of callosal axons of superficial layer neurons to the contralateral hemisphere in the developing neocortex. Conversely, forced expression of HP1γ facilitated axonal and dendritic development, suggesting that the increase of HP1γ is a rate limiting step in neuronal maturation. These results together demonstrate an important role for HP1γ in promoting axonal and dendritic development in maturing neurons.

(COI: No)

P1-101

Cell-type specific intracellular calcium recording in the nucleus accumbens in freely moving mice

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The nucleus accumbens (NAc), which largely receives the dopaminergic projection from the ventral tegmental area, plays a crucial role for the motivational reward and aversive response. The NAc is mainly constituted by two types of cellular groups, D1R- and D2R-MSNs (dopamine type1 and 2 receptor expressing medium spiny neurons), with differential functions and projections. To understand the function of NAc as a reward and aversive system, it is required to describe the behavior of individual cellular groups separately. However, it has been technically difficult because D1R- and D2R-MSNs are randomly distributed in the NAc. To elucidate the behavior of each cellular groups in the NAc, we developed a new fiber optical recording system for measurement of the intracellular calcium levels in deep brain structures. To express a calcium indicator in D2R-MSNs highly and specifically, we used the Knockin-mediated ENhanced Gene Expression system (KENGE-tet system; Tanaka *et al.*, 2012, *Cell Rep.*). The ratiometric calcium measurement using a forster resonance energy transfer (FRET)-based GECI, Yellow Cameleon-Nano 50, brought signals with high precision as a cancellation of artifacts by body motions of mice. We will report the particular patterns of calcium transients in the D2R-MSNs in the NAc by multiple aversive stimuli such as tail-suspension and restriction in mice.

(COI: No)

P1-102

Postnatal Development of Dendritic Structures in the Medial Prefrontal Cortex of the Marmoset

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In the primate cerebral cortex, dendritic spines rapidly increase in number after birth up to infancy or mid-childhood, and then decrease towards adulthood. Abnormalities in these processes accompany several psychiatric disorders. In this study, we examined developmental changes of basal dendrites and spines of layer III pyramidal cells in the medial prefrontal cortex (mPFC) of the common marmoset. The mPFC consists of several areas with distinct features in layer organization, histochemistry, connections, and, in humans, vulnerability to psychiatric disorders. We selected three areas for examination: granular dorsomedial prefrontal (area 8B/9), dysgranular ventromedial prefrontal (area 14r), and agranular anterior cingulate (area 24) cortices. Dendritic field areas, lengths, number of branching points, and total spine number reached a peak at 2-3 postnatal months in all three areas. However, the profiles of spine formation and pruning differed across the three areas with different degrees of granularity; the amount of spine loss from the peak to adulthood was less in areas 24 (33%) and 14r (29%) than in area 8B/9 (43%). Disturbance of this modest spine pruning in the less granular cortical areas may lead to an excessive loss of spines reported for areas 24 and 14r of schizophrenic patients.

(COI: No)

P1-103

Development of a multi-electrode array system for evaluation of human synaptic functions in neuron/astrocyte co-culture derived from human neural stem/progenitor cells

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A multi-electrode array (MEA) system enabled us to investigate synaptic functions in rodent brain slices and rodent neuron/astrocyte co-culture. It was, however, challenging to apply the human neurons/astrocytes to the MEA system, because it is not easy to prepare functional human neurons/astrocytes with simple methods. In this study, we utilized human fetal hippocampal neural stem/progenitor cells, HIP-009 cells to develop a novel MEA assay system; HIP-009 cells can differentiate into both neurons and astrocytes at an about equal ratio in the same culture. We observed that frequency and amplitude of spontaneous firings of differentiated HIP-009 cells were increased in a differentiation-time dependent manner. The electrophysiological maturation of neurons was promoted by supplementation of rat astrocyte-conditioned medium. Further analyses by using blockers for postsynaptic receptors (GABAzine, MK-801, and NBQX) revealed that the detected firings were resulted from the formation of functional synapses throughout differentiated HIP-009 neurons. In conclusion, we developed the novel in vitro assay system to evaluate human synaptic functions in mass cultures containing human astrocytes by utilizing HIP-009 cells in combination with the MEA system.

(COI: No)

P1-104

Augmentation of NMDA component of spinal monosynaptic reflex by high frequency stimulation in newborn rat

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The susceptibility of synaptic transmission to the stimulation frequency is a characteristic feature of immature animals. In an isolated spinal cord preparation of newborn rat, monosynaptic reflexes (MSRs) evoked by dorsal root stimulation, were mediated by both NMDA and AMPA glutamate receptors. In normal conditions, MSRs were constant in amplitude at 1/15 sec, and were completely eliminated by CNQX, which suggested normal MSRs were dependent on AMPA receptor. When stimulus rate was increased to 1/sec, MSR amplitudes were greatly reduced initially, and recovered later. This recovery of MSR was eliminated by APV, which suggested this recovery was dependent on NMDA receptor. In the presence of APV, AMPA component of MSRs were depressed and not recovered at 1/sec, which was presumably due to AMPA receptor desensitization. In the presence of CNQX and 0-Mg, NMDA component of MSRs were depressed initially and recovered, even though the stimulus rate was maintained at 1/sec. The stimulus intensity to induce this recovery, had to be strong enough to activate thin-fibers. This recovery was eliminated by application of spantide (16 μM), a non- specific tachykinin antagonist. It is suggested that thin sensory fibers can enhance Ia monosynaptic transmission through tachykinin receptors. Subtype of tachykinin receptors will also be discussed.

(COI: No)

P1-105

1, 8- and 1, 4-cineole presynaptically enhance spontaneous excitatory transmission in adult rat superficial dorsal horn neurons in a manner different from each other

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We have previously reported that 1, 8- and 1, 4-cineole, present in essential oils derived from eucalyptus, repeatedly increase the spontaneous release of L-glutamate onto spinal lamina II (substantia gelatinosa; SG) neurons with IC_{50} values of 3.2 and 0.24 mM, respectively, in a manner resistant to a voltage-gated Na^+-channel blocker tetrodotoxin. The present study examined a detail of the cineole actions by applying the patch-clamp technique to the SG neurons of adult rat spinal cord slices. The 1, 8-cineole activity was inhibited by TRPA1 antagonists, HC-030031 and mecamylamine, the latter of which is also known to be a nicotinic acetylcholine-receptor antagonist, but not by a TRPV1 antagonist capsazepine. On the other hand, the 1, 4-cineole activity was depressed by capsazepine but not by HC-030031 and mecamylamine. A TRPM8 antagonist BCTC, which inhibited sEPSC frequency increase produced by its agonist (-)-menthol, had no effect on the 1, 8- and 1, 4-cineole activities. 1, 8- and 1, 4-cineole reduced monosynaptically-evoked primary-afferent C-fiber but not A δ -fiber EPSC amplitudes, as with a TRPV1 agonist capsaicin and a TRPA1 agonist cinnamaldehyde, albeit with extents smaller than those of the agonists. It is concluded that the 1, 8- and 1, 4-cineole activities are mediated by TRPA1 and TRPV1 channels, respectively. This difference between the structural isomers of cineole may serve to know the property of TRP channels in the SG.

(COI: No)

P1-106

Effect of thymol on glutamatergic spontaneous excitatory transmission in adult rat spinal substantia gelatinosa neurons

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Transient receptor potential (TRP) channels expressed in the peripheral and central terminals of dorsal root ganglion neuron are involved in nociception, but the properties of TRP channels in the central terminal have not been fully examined yet. In order to know the properties of the central terminal TRP channels, we examined the actions of thymol, one of aroma-oil chemicals contained in thyme, on glutamatergic spontaneous excitatory synaptic transmission in lamina II (substantia gelatinosa; SG) neurons in adult rat spinal cord slices by using the patch-clamp technique. Superfusing thymol (1 mM) for 3 min increased the frequency of spontaneous excitatory postsynaptic current (sEPSC) with a minimal increase in its amplitude in almost all neurons examined. Seventy-eight % of the neurons also produced an outward current at -70 mV. These thymol activities were repeated at a time interval of 30 min and resistant to a voltage-gated Na^+-channel blocker tetrodotoxin. The sEPSC frequency increase was inhibited by a TRPA1 blocker HC-030031 but not a TRPV1 blocker capsazepine, while these blockers had no effect on the outward current. It is concluded that as with eugenol and carvacrol, thymol increases the spontaneous release of L-glutamate onto SG neurons by activating TRPA1 channels while producing an outward current in SG neurons without TRPA1 and TRPV1 activation. This result could serve to know the properties of central terminal TRP channels.

(COI: No)

P1-107

Localization of kirrel3 protein at synaptic sites in the mouse cerebellum

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A member of the immunoglobulin superfamily, kirrel3, plays important roles in the axonal fasciculation of the specific olfactory sensory neurons as well as in the axonal coalescence of the specific vomeronasal sensory neurons. In the brain of adult mice, kirrel3 interacts with the synaptic scaffold protein, calcium/calmodulin-dependent serine protein kinase, indicating the possible involvement of kirrel3 in synaptic function. Previously, we have reported that the kirrel3 gene was widely expressed in the cerebellum including the granule cells, Purkinje cells, and interneurons during development. In the present study, we investigated the localization of kirrel3 protein in the postnatal and adult cerebellum using immunohistochemistry. In the cerebellum, the expression of kirrel3 protein was first observed in the internal granule cell layer (IGL) at postnatal day (P) 7 and reached a maximum at P14. From P7 to P28, kirrel3 was colocalized with PSD95 at synaptic sites of IGL. From P21 to P70, the expression of kirrel3 protein was also observed in the PSD95-positive nerve plexus of basket cells (pinceau), which surrounds the axon initial segment of Purkinje cells. These findings suggest that kirrel3 may be involved in the synaptic formation/plasticity in the cerebellum during postnatal and adult stages. This work was supported by a Grant-in-Aid for Scientific Research (B) from Japan Society for the Promotion of Science (22390036).

(COI: No)

P1-108

Interleukin-18 knock out mouse induced degeneration of mitochondria in the dentate gyrus of the hippocampus

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Interleukin-18 is thought to regulate motor activity and spatial learning, and mediate inhibition of LTP in the dentate gyrus of the mouse. We investigated whether morphological changes have occurred in the dentate gyrus of the Interleukin-18 knock-out mouse (12 weeks old) by using the electron microscope. In the molecular layer, many degenerated mitochondria were found and located in the axon terminals. They were round, small (about 0.3 μm in diameter), electron dense, and showing indistinct structure of crista. The terminals containing degenerated mitochondria were small (about 0.7 μm width), and attached slender dendrites (about 0.6 μm width). These terminals contained round or pleomorphic synaptic vesicles and formed asymmetric synaptic contacts. The number of terminals contained degenerated mitochondria was smaller in the inner part than those in the outer part of the molecular layer. The granule cells were round and similar to those of the wild type mouse. In the polymorphic layer, there were a few small terminals containing degenerated mitochondria, whereas the large terminals of the mossy fibers contained non-degenerated mitochondria. These results suggest that morphological and physiological changes occur at the axon terminals of the entorhinal-dentate gyrus projections in the Interleukin-18 knock-out mouse.

(COI: No)

P1-109

Social isolation during critical period causes reduced excitatory inputs onto mouse medial prefrontal cortex neurons in adulthood

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Social experience is crucial for the functional development of medial prefrontal cortex (mPFC). Rearing mice in social isolation produces hypomyelination of mPFC in adulthood, which is paralleled by behavioral impacts including poor sociality (Makinodan et al., 2012). However, little is known about the alteration in mPFC neural circuits induced by social isolation. We studied the effects of social isolation on excitatory synaptic inputs onto layer 5 pyramidal cells of mouse mPFC in adulthood. The mouse was reared in isolation for two weeks (P21-35: early isolation or P35-49: late isolation), and then returned to its home cage and reared with its littermates. Whole-cell recordings were performed using slices prepared from P63-67. We found that the spontaneous excitatory postsynaptic current (sEPSC) frequency and miniature excitatory postsynaptic current (mEPSC) frequency were significantly lower in early-isolated mice than in grouped mice. However, there was no significant difference in sEPSC and mEPSC frequencies between late-isolated mice and grouped mice. These results show that only 2 weeks social isolation from weaning reduces excitatory synaptic inputs onto layer 5 pyramidal cells in mPFC and suggest that social experience during the critical period is pivotal in the development of mPFC excitatory neural circuit.

(COI: No)

P1-110

Pairs of stimuli enhance cell firing in hippocampal CA1 area

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In hippocampus, synaptic efficacy is regarded as the neural basis for learning and memory. To understand the mechanism of these, it is necessary to test how information is integrated in hippocampal CA1. Hippocampal CA1 neurons receive inputs from entorhinal cortex indirectly via a trisynaptic path, in which CA3 Schaffer collaterals (SC) form synapses on proximal CA1 dendrites in stratum radiatum (SR). CA1 neurons also has excitatory connections directly with entorhinal cortex via the perforant path (PP). These direct inputs synapse on distal pyramidal neuron dendrites in stratum lacunosum moleculare (SLM). The trisynaptic path has a longer delay time so that information arising from entorhinal cortex arrives at SLM 10-20 ms prior to the arrival of information at SR in CA1. The functional role of the direct PP inputs is not well understood, although recent research indicates that these inputs have important effects on CA1 pyramidal cells. In this study, the effects of interactions between PP and SC inputs on field EPSP (fEPSP) in CA1 area in brain slice preparation of rat are investigated. We simultaneously recorded population spike from stratum pyramidale and fEPSPs from SR in CA1, extracellularly. The results indicate that synaptic plasticity is modulated by different pairing intervals. In addition to this, we used GABAA receptor antagonist to test whether this modulation relies on inhibitory circuits.

(COI: No)

P1-111

Regulation of neuritogenesis in PC12 cells by temperature-controlled repeated thermal stimulation

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This study aimed to examine the regulation of neuritogenesis (NG) by temperature-controlled repeated thermal stimulation (TRTS) in rat neuron-like PC12 cells. Plated PC12 cells in growth or differentiation medium were exposed to TRTS using a heating palte (HP) (preset surface temperature of the HP, 39.5℃ or 42℃) for up to 18 h/day. This was followed by an evaluation of alternations in cell growth, extent of NG, or acetylcholinesterase (AChE) activity (a neuronal marker). To analyze the mechanisms underlying the effects of TRTS on these cells, its effects on intracellular signaling were examined using: the TrkA inhibitor GW441756, PKA inhibitor H89, p38 MAPK inhibitor SB203580, and MEK inhibitor U0126. While the TRTS of 39.5℃ did not decrease the growth rate of cells in the cell growth assay, it increased the number of neurite-bearing PC12 cells and AChE activity without addition of other inducers of NG. Furthermore, U0126, SB203580, and H89, but not GW441756, considerably inhibited TRTS-induced NG. These results suggested that the TRTS could induce NG and that activation of both the ERK1/2 and p38 MAPK pathways is required for the mechanism of TRTS-dependent NG in PC12 cells.

(COI: No)

P1-112

Morphological analysis of Purkinje cell-specific calcineurin B1 subunit KO mice

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A Ca^{2+}/calmodulin-dependent protein phosphatase Calcineurin (CN) is widely expressed at the central nervous system and plays important roles in various neuronal functions, such as synaptic transmission and the expression of long-term synaptic plasticity. However, little is known about how CN is associated with the formation of excitatory and inhibitory neuronal network. In the present study, we produced a novel mouse line CNB1-PCKO mouse which lacks a regulatory subunit of CN, CNB1, specifically in cerebellar Purkinje cells (PCs) and investigated the cerebellum by morphological techniques. In light microscopic analysis using with immunofluorescence, CNB1-PCKO mice showed that climbing fiber (CF) territory was proximally retracted. By neurotracing technique, some CFs showed aberrant PC wiring and caused multiple CF innervation at proximal PC dendrites. At the electron microscopic level, VIAAT-positive inhibitory terminals frequently formed asymmetrical synapses with PC spines, which in normal adult cerebellum are innervated by excitatory terminals. Postembedding immunogold microscopy revealed that such atypical inhibitory synapses on PC spines expressed both AMPA and GABAA receptors on the postsynaptic membrane. In typical PC spines contacting parallel fiber terminals, the density of AMPA receptor was significantly increased in CNB1-PCKO mice. These result suggest that CNB1 in PCs is essential for CF-PC mono-innervation, anatomical targeting of PC spines to excitatory terminals, and limiting AMPA receptor content at excitatory synapses.

(COI: No)

P1-113

Optical mapping of vagus nerve-related brainstem nuclei in the mouse embryo

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The vagus nerve (N.X) transfers autonomic input and output information to and from the brainstem, and analysis of the N.X-related brainstem nuclei is the first step to understand the functional organization of the autonomic neuronal circuits. Investigations of the neural network organization have been hampered because conventional electrophysiological means have some technical limitations. In the present study, the multiple-site optical recording technique with a voltage-sensitive dye was used to survey the functional organization of the vagal system in a mouse embryo. Stimulation of the N.X in E11 to E14 mouse embryos elicited optical responses in areas corresponding to the vagal sensory and motor nuclei. Postsynaptic responses in the first-order sensory nucleus, the nucleus of the tractus solitarius (NTS), were identified from E11, suggesting that sensory information was transferred to the brain by this stage. In addition to the NTS, optical responses were identified in the rostral and contralateral brainstem regions, which appeared to correspond to second/higher-order nuclei of the vagus nerve. Postsynaptic responses in the second/higher-order nuclei were detected from E12, suggesting that polysynaptic pathways were functional by this stage. We discuss the results of optical mapping, comparing them with previous findings obtained in chick and rat embryos.

(COI: No)

P1-114

Roles of BMP4 signaling in synapse development

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Synapse development is a process precisely regulated by both genetic programs and activity-dependent processes. Proper remodeling of synapses is required for refinement of neuronal circuits. The process of synapse turnover must be regulated by specific signaling mechanisms. BMPs are members of TGF-β superfamily. Secreted BMPs exert their functions through activation of heterotetrameric complex of BMPRI and BMPRII. After ligand binding, BMPRII phosphorylates a cytoplasmic domain of BMPRI. This phosphorylation activates BMPRI and initiates subsequent phosphorylation of the intracellular signaling molecules. Recent studies indicate a role for BMP4 signaling pathway in development of Drosophila neuromuscular junction and neural networks in the mammalian cerebellum and brain stem. Furthermore, the expression level of BMP4 in the mammalian CNS was shown to be related to learning and memory. These results indicate that BMP4 may regulate the process of synapse development and its activity-dependent modulation. Here we report that BMP4 has a function for regulating the stability of synaptic structures. We studied the roles of BMP4 in the process of synapse development by visualizing dendritic structure and distribution of synaptic molecules in hippocampal cultures taken from BMP4 conditional KO mice. Furthermore, live imaging analyses revealed spatiotemporal regulation of BMP4 exocytosis. These results suggest that BMP4-dependent mechanism of synapse remodeling is essential in proper formation of neural network in the mammalian CNS.

(COI: No)

P1-115

Dendritic spine dynamics during growth if hippocampal slice culture

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The organotypic slice culture of brain has many experimental advantages, among which is the possibility of pursuing the morphological changes consecutively for long periods. We previously analyzed the dynamics of dendritic spines in the stable culture of the mouse hippocampus after repetitive inductions of chemical LTP that led to a slowly developing long-lasting synaptic enhancement. The spines are in a stochastic equilibrium between generation and retraction. The plasticity-producing stimulus increased fluctuation keeping the equilibrium at first and then biased the equilibrium toward generation to result in a net increase in spine number. Here we analyzed the dendritic spine dynamics before maturation of culture to know whether or not the developmental synapse formation follows a course similar to the above-mentioned post-maturational (i.e. plasticity-related) synapse formation. We found that the number of spines increased through a biased fluctuation where the rate of retraction was lower than that in the mature culture. This dynamics should not be an artifact of culturing, since the cultures prepared from younger mouse pups behaved not in a culture-day-matched manner but in a cell-age-matched manner. Joro spider toxin, a blocker for the calcium-permeable AMPA receptor that is expressed in the developing hippocampus, suppressed the net spine increase through raising the rate of retraction. This work is supported by Kaken-hi to A.O.

(COI: No)

P1-116

A potential *in vitro* model system of the stress-associated memory disorder

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Previously we found in the organotypic slice culture of the rodent hippocampus that three repeated inductions, but not a single induction, of chemical LTP (cLTP) led to a slowly developing long-lasting synaptic enhancement coupled with new synapse formation. Naming this structural plasticity phenomenon RISE (*R*epetitive LTP-*I*nduced *S*ynaptic *E*nhancement), we propose that it should serve as a model system for analyzing the cellular processes underlying memory consolidation. In this study, we analyzed the effects of externally applied glucocorticoid as mimicry of stress *in vivo*. Dexamethasone (Dex; 1-100nM), when applied for a 24h period beginning 12h after the third cLTP induction, suppressed the increase in the density of dendritic spine CA1 pyramidal neurons. The analyses of spine dynamics revealed that Dex suppressed the elevation of spine generation rate that occurred in during RISE development. Dex also suppressed the enhancement of electrophysiologically-monitored strength of CA3-CA1 synapse. Dex did not induce neuronal death by this dose and period. The Dex's effect was reversed by mifepristone, a glucocorticoid receptor antagonist. Mineralocorticoid aldosterone (10nM) failed to interfere with RISE. These results endorse the usability of this *in vitro* system for analyzing the cellular mechanisms underlying the stress-associated memory disorder. This work was supported by Kaken-hi to A.O.

(COI: No)

P1-117

Cholinergic modulation of GABAergic synaptic transmission in the dorsal raphe serotonin neurons

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The dorsal raphe nucleus (DRN) is the origin of central serotonin (5-HT) system, plays an important role in the regulation of many physiological processes such as sleep/arousal, food intake and mood. The DRN has been thought to be subdivided into several clusters on the basis of differences in cellular morphology, expression of neurotransmitters such as 5-HT, dopamine, GABA and glutamate. Among these, there are many reports that GABA synapses play roles for regulation of excitability of 5-HT neurons by a form of feedback inhibition. However, the modulatory effects on GABAergic synapses at the DRN 5-HT neurons are poorly understood. In this study, we investigated modulatory effects of cholinergic receptor on GABAergic synapses in the mouse (C57/BL6, postnatal days 35-50) DRN 5-HT neurons using whole-cell recordings in the brain slices. Muscarinic receptor agonists, muscarine and carbachol decreased the amplitude of stimulation-evoked IPSCs (eIPSC) with an increase in the paired-pulse ratio, and their effect was reversibly abolished by a M2-receptor antagonist, AFDX-116. Based on these results, the activation of M2 receptor is suggested to be responsible for presynaptically decreasing the amplitude of eIPSCs. We next examined whether endogenous acetylcholine (ACh) affected GABAergic transmission in the DRN neurons. Conditioning stimulation at pedunculo pontine tegmental nucleus which has many cholinergic neurons decreased the amplitude of eIPSCs. These results suggest that the excitability of DRN 5-HT neurons may be positively controlled by disinhibition manner of GABAergic transmission.

(COI: No)

P1-118

Ischemia-induced potentiation of cortical responses to hindpaw stimulation is partly mediated by nitric oxide at the spinal cord level

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We frequently experience postischemic tingling sensation. Ischemia also produces nerve conduction block that may modulate spinal neural circuits, and tingling sensation may be induced as a result. In a mouse model, reduced mechanical thresholds for hindpaw-withdrawal reflex were reproduced after a high pressure was applied around the hindpaw. Neural activities in the spinal cord and the primary somatosensory cortex (S1) were investigated using flavoprotein fluorescence imaging. Ischemic treatment induced potentiation of the ipsilateral spinal and contralateral S1 responses to hindpaw stimulation. We also found that S1 responses elicited by vibratory stimulation applied to the hindpaw contralateral to the ischemic treatment were significantly potentiated during ischemia, suggesting that some diffusible mediators were involved in the potentiation. Nitric oxide (NO) is one of such diffusible mediators involved in synaptic potentiation. We applied L-NAME, an inhibitor of nitric oxide synthase, intrathecally at the L5-L6 intervertebral space, and the potentiation of S1 responses elicited by vibratory stimulation applied to the hindpaw contralateral to the ischemic treatment was clearly inhibited. These results suggest that NO has some roles at the spinal cord level in the induction of postischemic potentiation.

(COI: No)

P1-119

Otoferlin alters mode of exocytosis at the mouse inner hair cell ribbon synapse

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Sound encoding depends upon Ca^{2+}-mediated exocytosis at the inner hair cell (IHC) ribbon synapse in the cochlea. Otoferlin, a multi-C2 domain protein, has been proposed to regulate Ca^{2+}-triggered exocytosis at this synapse, but the precise mechanisms of otoferlin function remain unclear. In this study, we performed whole-cell patch-clamp recordings of excitatory postsynaptic currents (EPSCs) from postsynaptic spiral ganglion neurons (SGNs) in otoferlin mutant mice, in order to investigate the effect of otoferlin disruption at individual synapses with single release event resolution. Otoferlin deletion dramatically decreased the rate of spontaneous release and high potassium-evoked release, suggesting disrupted stimulus-secretion coupling in IHCs. A missense Otoferlin mutation (pachanga) also reduced the release rate but spared stimulus-secretion coupling. These findings support the proposed roles of otoferlin in Ca^{2+} sensing for fusion and vesicle supply. While both otoferlin mutant SGNs showed a decrease in the mean EPSC amplitude, large-sized and variable-shaped EPSC remained present despite the massively reduced rate of release. In addition, both otoferlin mutant SGNs exhibited a smaller fraction of multiphasic EPSCs. These findings argue for uniquantal release at the IHC ribbon synapse (Chapochnikov, Takago et al. (2014) Neuron 83:1389-1403), and we suggest a role of otoferlin in regulating the dynamics of vesicle fusion pore.

(COI: No)

P1-120

Input-selective expression of glutamate receptor GluD1 at ascending somatosensory pathway synapses in the ventral posteromedial thalamic nucleus

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Of the two members in the δ family of ionotropic glutamate receptors, GluD1 is widely expressed in higher regions of the adult brain. We have recently demonstrated that GluD1 works in concert with GluD2 for the construction of cerebellar synaptic wiring through synapse-connecting function (Konno et al., 2014). However, little is known to date regarding the expression and function of GluD1 outside the cerebellum. To address this issue, we examined the expression in the ventral posteromedial thalamic nucleus (VPM), a relay station in the trigeminal somatosensory pathway. Double-labeling fluorescence in situ hybridization addressed that GluD1 mRNA was expressed in glutamatergic thalamic neurons expressing vesicular glutamate transporter VGluT2 mRNA. VPM neurons are known to receive two types of glutamatergic inputs, one from VGluT2-positive ascending inputs from the brainstem trigeminal nuclei and another from VGluT1-positive descending inputs from the somatosensory cortex. By immunofluorescence, GluD1-positive clusters were closely apposed to VGluT2-positive terminals, but not VGluT1-positive terminals. Postembedding immunoelectron microscopy revealed that GluD1 was selectively localized on the postsynaptic membranes of dendritic protrusion surrounded by VGluT2-positive terminals. Thus, GluD1 displays input-selective expression in VMP thalamic neurons, and is selectively localized to their ascending pathway synapses.

(COI: No)

P1-121

Formation of ectopic synapses at retina in presynaptic active zone protein CAST/ELKS deletion mutant

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A photoreceptor cell is a specialized neuron which converts light into signals in the retina. The two types of cells, rods and cones, form synapses at a band known as the outer plexiform layer. In old retina, these synapses are ectopically localized in the outer nuclear layer (ONL), which may cause the loss of function. In young retina, this ectopic localization is found in some deletion mutants such as Bassoon and CAST. Here we explored the effect of the deletion of ELKS, a family member of CAST, and the deletion of both on the localization and structure of ribbon synapse and the visual processing in retina. The ELKS conditional knock out (KO) under the control of *Crx* promoter showed normal development and less effect on the mislocalization of the synapses. However, CAST and ELKS double KO (dKO) showed drastic aberrant synapse formation into the ONL. To know how the structural alteration affects the signal transduction in retina, we measured the gain of eye movement with the optokinetic response. From this test, we found serious gain reduction in dKO, however the gain was detectable indicating that the dKO was not the complete blindness. From these results, we speculate that CAST and ELKS contribute to the maturation of retinal ribbon synapse structurally and functionally.

(COI: No)

P1-122

BRAG2c, a long C-terminal splice variant, interacts with endophilin III to mediate AMPA receptor internalization

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Brefeldin A-resistant Arf-GEF 2 (BRAG2) is a guanine nucleotide exchange factor (GEF) that selectively activates ADP ribosylation factor 6 (Arf6). Arf6 is known as a small GTPase that regulates membrane trafficking between plasma membrane and endosomes. It has been reported that BRAG2 directly binds to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type glutamate receptors (AMPARs), and is involved in the synaptic long-term depression by regulating the endocytosis of AMPARs at hippocampal excitatory synapses. However, the molecular mechanism mediating between AMPAR endocytosis and BRAG2-Arf6 signaling remains to be elucidated. Here, we report that a long C-terminal splice variant, BRAG2c is highly enriched in the postsynaptic density (PSD) fraction compared to a short C-terminal splice variant, BRAG2b, and selectively localized at the excitatory PSD accompanied by colocalization with AMPARs in the adult mouse brain. Using yeast two-hybrid and immunoprecipitation assays, we show that BRAG2c interacts with PDZ domain of PSD-95 and SH3 domain of endophilin III. Furthermore, the blocking of the interaction between BRAG2c and endophilin III disturbed the endocytosis of AMPARs triggered by mGluR-signaling in the hippocampal primary culture neuron. Taken together, these findings unveil a novel molecular mechanism by which the BRAG2-Arf6 signaling regulates the synaptic AMPARs through the interaction with BRAG2 and endophilin III.

(COI: No)

P1-123

Sevoflurane suppresses presynaptic calcium influx leading to inhibition of excitatory neurotransmission at the hippocampal CA1 synapses

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Despite diverse effects of volatile anesthetics in the brain have been studied extensively, little is known about the effect on excitatory neurotransmission. In this study, we examined the effect of sevoflurane (Sev), one of the major volatile anesthetics, on excitatory synaptic transmission in hippocampal CA1 region. Transverse hippocampal slices were made from mice of 9 - 37 days old. Field excitatory postsynaptic potential (EPSP), paired-pulse ratio (PPR), presynaptic fiber volley (FV) were measured with extracellular recordings. In addition, fluorescent measurement of presynaptic calcium influx was used to investigate the mechanisms of presynaptic action of Sev. Sev at 5 % were mixed with 95% O_2 and 5% CO_2 and bubbled in artificial cerebral spinal fluid. Application of Sev reduced the amplitude of field EPSP to 45 % ± 8 % of control (n = 5). This effect was accompanied with concurrent enhancement of PPR to 127 % ± 5 % of control (n = 12), suggesting possible presynaptic site of action of Sev. The amplitude of presynaptic FV was not significantly affected by Sev. In contrast, fluorescent measurements revealed that presynaptic calcium influx was suppressed by Sev to 69 % ± 6 % of control, and simultaneously recorded EPSP to 44 % ± 2 % of control (n = 7). These results suggest that Sev potently suppresses excitatory synaptic transmission without affecting presynaptic action potential, but possibly due to inhibition of presynaptic voltage-gated calcium channels.

(COI: No)

P1-124

Sema7A-PlxnCl signaling is essential for triggering activity-dependent synapse formation in the mouse olfactory bulb

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Odor information detected by olfactory sensory neurons is transmitted to the brain through second-order neurons, the mitral/tufted cells. Here we report that a pair of signaling molecules, Sema7A expressed by olfactory sensory neurons and its receptor PlxnC1 expressed by mitral/tufted cells, are essential for triggering synapse formation in the olfactory bulb. In both knockout mice for either Sema7A or PlxnC1, not only synapse formation but also dendrite maturation is perturbed. The same phenotype is also observed in the knockout of cyclic nucleotide gated channels. Surprisingly, this phenotype of dendrite maturation in the channel knockout is rescued by the forced expression of Sema7A alone with the odorant receptor promoter. We can therefore conclude that Sema7A-PlxnCl signaling plays a key role in triggering the activity-dependent synapse formation and dendrite selection of mitral/tufted cells in the olfactory bulb.

(COI: No)

P1-125

c-Src dependent cell polarity in fibroblasts cultured on adhesive micropatterns

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Focal adhesions (FAs) and associated stress fibers (SFs) are specialized components contributing to cellular events such as cell migration, wound healing, adhesion of cells, etc. FAs recognize the boundary between the plasma membrane and specific extracellular matrix proteins and are involved in cell orientation and polarity. Although fibroblastic cells select specific substrates for typical cell-substrate adhesion, the mechanisms that regulate orientation and polarity are not clear. In this study, using adhesive micropatterns (MPs) in order to regulate polarized cell spreading, together with c-Src inhibitors (Src inhibitor No. 5, Biaffin), we analyzed the behavior of cultured fibroblasts during the organization of cell polarity on adhesive MPs. When normal fibroblasts attached to the MPs (width; 10 or 15 μm), phosphorylated c-Src (pY418), the active form of c-Src, was intensely detected along the inner border between the adhesive MPs and non-adhesive glass surface, reflecting the active c-Src location at the inner border. When cells were treated with c-Src inhibitor, cells were significantly elongated compared to normal cells, and aligned along the longitudinal axis of the MPs in a spindle shape with well developed SFs. However, staining of phosphorylated c-Src was not detected at the border of the MP and non-adhesive glass surface. These observations suggest that the activation of c-Src plays a key role in the recognition of the border between the adhesive MP and non-adhesive glass surface. Moreover, inactivation of c-Src causes polarized elongation of cells.

(COI: No)

P1-126

N-terminus of paxillin regulates actin stress fiber formation by binding to the active Fyn

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Rho-kinase (ROK)-mediated actin stress fiber formation plays important roles in many cellular functions, including cell adhesion and motility. We previously found the involvement of Fyn tyrosine kinase as an upstream molecule of ROK in actin stress fiber formation. However, the molecular mechanisms between Fyn and ROK have not been clarified yet. To search for the downstream molecule of Fyn, we performed pulldown assay with HaloTag constitutively active Fyn (CA-Fyn) and dominant negative Fyn (DN-Fyn) in human vascular smooth muscle cells (VSMCs), and obtained the candidate molecules which selectively bind to CA-Fyn, but not to DN-Fyn. Subsequently, matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) enabled us to identify paxillin as a novel downstream molecule of Fyn. To clarify binding site of CA-Fyn on paxillin, the recombinant Fyn and paxillin were obtained in baculovirus and E.coli expression system respectively. Surface plasmon resonance assay showed that CA-Fyn bound to N-terminus, but not C-terminus of paxillin, while DN-Fyn bound to neither of them. Colocalization of CA-Fyn and N-terminus of paxillin during the stress fiber formation in VSMCs further confirmed their binding. In addition, the overexpression of N-terminus of paxillin inhibited the actin stress fiber formation. Taken together, these results demonstrate that paxillin, as a novel signal mediator, regulates actin stress fiber formation by N-terminus binding to the active Fyn.

(COI: No)

P1-127

The morphorogical role of myosin light-chain kinase to form podosome in smooth muscle cell

Tanaka, Hideyuki; Nakakura, Takashi; Nishijima, Yoshimi; Arisawa, Kenjiro; Asano, Anshin Hoshino; Kiuchi, Yoshiko; Hagiwara, Haruo (*Sch. Med. Teikyo Univ*)

We revealed that Myosin light chain kinase (MLCK) has been essential to form podosome. Podosome is intracellular structure which is actin-filaments as a core. Vascular smooth muscle cell (VSMC) in the culture dose not little, if any, form the podosome, but it includes the protrusion by adding phorbol 12, 13 dibutyrate (PDBu) as chemical mediator driven Protein kinase C. We added PDBu to the cultures of A7r5 cells of line of VSMC as shown by the immunno-fluorescent micrograph. At first, We observed that PDBu had been applied to the medium culturing A7r5 cells by the use of the fluorescent microscope. After incubation for specified periods, the culture plates were subjected to the count how many cells formed podosome. The numbers of VSMC with podosome were increased with the elapse of time. The VSMCs with podosomes were maximal within 30 min, and ～90 % cells developed podosomes. The scanning electron micrographs were shown that the cellular surface A7r5 cell when stimulated by PDBu, the cellular surface protruded podosomes; higher magnification showed that they were composed of the legs connecting the ball-like feets to the cellular surface of A7r5. Transmission electron micrographs of the vehicle and the PDBu-stimulated cells were shown, respectively. Upon the stimulation by PDBu, the tracks of actin- bundles were disrupted, and most of them were capped by the electron dense plaque, a capping that had been not penetrated by the actin-bundle. Taken together we proposed the idea that the dense plaque had been feet, and actin-bundle been legs.

(COI: No)

P1-128

Structural change of myosin head in skeletal muscle fiber without thin filament

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Myosin converts chemical energy of ATP to mechanical work in combination with actin. The molecular mechanism of this chemo-mechanical transduction is still unknown, mainly because mechanical work significantly deforms the molecules. Therefore, it is of interest to follow the intrinsic structural changes of myosin in the absence of actin. The changes would depict a conformational path of minimal potential. We here followed intrinsic structural changes of myosin heads in sarcomere where conformational freedom of myosin would be highly restricted in a range optimized for the physiological path unlike in the purified solution system. Actin was removed from sarcomere of skinned fibers with gelsolin treatment, and helically arranged myosin heads were observed with X-ray diffraction (at BL6A of PF) following the ATP hydrolysis steps of M, M-ATP, M-ADP-Pi, M-ADP, and M, where M represents myosin. Compared with M and M-ATP (trapped with N-phenylmaleimide) states, myosin heads in M-ADP-Pi state were retracted close to the backbone of the thick filament. Retrograde binding of ADP to M to yield M-ADP did not cause this marked transition of myosin heads. Since orthograde conformational change at M-ADP state following Pi release is generally considered to be coupled with mechanical work of myosin, radial retraction and following protrusion of myosin heads would likely be the prime mover as in the case of crawling bristle grass in your gripping hand.

(COI: No)

P1-129

The effect of Nisin on keratinocyte cytoskeleton and intercellular junction

Kitagawa, Norio; Inai, Tetuichiro (*Fukuoka Dent. Coll., Fukuoka, Japan*)

Bacteriocins are proteinous antibacterial substances produced by bacteria. Nisin is bacteriocin produced by *Lactococcus lactis subsp. lactis*. Nisin is commonly used as a food preservative throughout the world. Bacteriocins such as nisin are thought to act only prokaryote cell and doesn't effect on normal eukaryote cell.

In this study, we investigated the effects of nisin to human epidermal keratinocyte cell line (HaCaT). Keratinocytes exposed to nisin continue to proliferate. Though, exposure of keratinocytes to nisin perturbed cobble stone-like structures. Detachment and fragmentation of the cells were increased by nisin exposure. Keratinocytes exposed to nisin showed nisin formed gaps or holes in the keratinocyte layer. As Nisin seemed to effect on cell-cell junction and cytoskeleton, we investigated the effect by immunofluorescence microscopy. Localization of desmosomal cadherin protein, desmoglein 3 (DSG3) and adherens junction protein, β-catenin was disrupted. Furthermore, intermediate filament protein, cytokeratin 5 and cytokeratin 17 decreased their localization at the cell boundary.

Taken together, our result indicates that nisin effects on not only prokaryote cells but also on normal eukaryote cells. Nisin seems safe from long history of successful usage as a food preservative. However, it doesn't mean nisin doesn't effect on eukaryote cell.

(COI: No)

P1-130

Molecular dissection of intracellular neurofilaments

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Neurofilaments (NFs) are intermediate filaments (IFs) expressed specifically in neurons and considered pertinent to support both of their morphological changes and functional structures. Our understanding of the NF dynamics in living cells, however, has been relatively poor compared to that of other cytoskeletal components.

To elucidate intracellular elementary polymerization processes, we monitored the behavior of NF medium protein (NF-M) heteropolymerizing with other IFs in cultured cells. Murine primary hippocampal neurons were infected with adenoviral vectors carrying green fluorescent protein tagged NF-M genes, and SW13vim(-) cells, which lack endogenous IFs, were coinfected with either of other viruses expressing NF light protein or α-internexin in addition to the tagged NF-M virus. We then performed fluorescence recovery after photobleaching experiments at several days after infection with highly inclined/laminated optical sheet and/or total internal reflection fluorescence microscopy, and observed different localities of NF polymerization, depending on the culturing age, cell species, and IF constituents. Our findings collectively suggest that the NF dynamics changes over time and space, reflecting the differentiation stages of neurons and their states. We will also discuss our newly developed novel microscopic methods which enable us to determine not only the protein positions but also the arrangements of NF polymers.

(COI: No)

P1-131

Mechanisms of CRMP2-induced GTP-state microtubule formation

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The asymmetric microtubule cytoskeleton is essential for axon-dendrite specification in developmental neurons and for polarized protein sorting in mature neurons. In axons of mature neurons, GTP-state microtubules (MTs) are enriched over GDP-state MTs and are preferentially searched for as landmarks by the conventional molecular motor Kinesin-1, which goes into the axon among many processes in neurons. Recently, we solved the cryo-EM structure of GTP-state MTs. They have the characteristic conformation at both the longitudinal and lateral contacts between tubulins, albeit those polymerized in vitro have the unstable lattice especially at the surface. We thus hypothesized that stable GTP-state MTs could be polymerized in vitro with the support by some MT-binding proteins/factors that we sought for. Among several candidates, CRMP2 plays the essential role at the early stages in the axonal development by promoting the axonal specification in cultured mammalian neurons. CRMP2 was also reported to interact with GTP-state tubulins and promote MT assembly in vitro. Considering that one of the functional landmarks in axons is an enrichment of GTP-state MTs, CRMP2 might promote the GTP-sate MTs in single process to give it the axonal signature, albeit the molecular mechanism of how CRMP2 induces the GTP-state MT formation is poorly understood. Here we characterize the role of CRMP2 in promoting the stable GTP-state MTs by using the several structure-based technics, such as X-ray crystallography, small angle X-ray scattering, cryo-electron microscopy, and fluorescence microscopy.

(COI: No)

P1-132

The elongation of primary cilia via the acetylation of α-tubulin in human fibroblast treated with lithium chloride

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Primary cilium is found on almost all types of cells in the human body and typically serves as the mechanical sensor of the cell. Lithium ion is known to promote the elongation of primary cilia in a variety of cell types, but it is unknown whether lithium is involved in the acetylation of α-tubulin which is important for the function of primary cilia. In order to reveal the relationship between the elongation of primary cilia by lithium and the acetylation of α-tubulin, we first observed the formation and structure of primary cilia in KD cells, a cell line deriving fibroblasts in human labium. Subsequently, by immunohistochemical and western blot analysis we elucidated that the length of primary cilia and acetylation of α-tubulin are regulated by lithium chloride (LiCl) in a time- and concentration-dependent manner. We next performed the RT-PCR, RNAi based experiments and biochemical study using an inhibitor of glycogen synthase kinase-3β (GSK-3β). We found that LiCl mobilizes the α-tubulin N-acetyltransferase 1 (αTAT1) in the signaling pathway mediating GSK-3β and adenylate cyclase III. In conclusion, LiCl treatment activates αTAT1 by the inhibition of GSK-3β and promotes the α-tubulin acetylation, and the acetylation of α-tubulin by αTAT1 facilitate the elongation of primary cilia.

(COI: No)

P1-133

Roles of a ciliary gene, Nphp2/Inv in cell cycle control

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Primary cilia are a hair-like membrane structure, presenting in almost every cell type. Disruption to the ciliary structure or its function causes multiorgan diseases known as ciliopathies such as situs inversus and cystic kidney disease. Increased BrdU incorporation has been described in cystic kidneys associated with ciliopathies. We previously reported that increased BrdU incorporation and abnormal mitotic axis formation are observed in the mutant Inv mice, and the S/G2/M population (with Fucci-Geminin positive) is increased in Nphp2/Inv-knockdown (KD) cells. Although cell cycle abnormality is well described in inv mutant, mechanisms to control cell cycle are unknown. To understand cell cycle abnormality observed in inv mutant mice, we mated inv mice with Fucci mice to analysis cyst lining cells in cell cycle. We are determining which stage in cell cycle is disturbed in Nphp2/Inv-knockdown (KD) cells. Inv /nephrocystin-2 is localized at the proximal potion of the renal cilia. However, its localization during cell cycle is controversial. We are analyzing localization of Inv /nephrocystin-2 using Fucci cells. The results will be presented.

(COI: No)

P1-134

DGKζ-interacting NAP1-like proteins regulate cell cycle and apoptosis by controlling p53 acetylation

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Diacylglycerol kinase (DGK) converts diacylglycerol to phosphatidic acid by phosphorylation. Since both of these lipids are regarded as key molecules in lipid signaling, DGK is considered to be an important regulator in lipid-mediated signal transduction. Of DGKs, DGK ζ , characterized by the presence of a nuclear localization signal (NLS), was shown to be localized to the nucleus in neurons and the transfected cells. We previously identified nucleosome assembly protein (NAP) 1-like 1 (NAP1L1) and NAP1-like 4 (NAP1L4) as novel DGK ζ interaction molecules. However, functional roles of NAP1Ls remain unknown. Since DGK ζ is shown to regulate p53 function, we examined how NAP1L1 and NAP1L4 regulate p53-mediated cell cycle and apoptosis. In WST-1 assay, knockdown of NAP1L1 promoted cell proliferation, whereas NAP1L4 knockdown inhibited cell cycle progression. In an apoptosis assay using DNA-damaging agent doxorubicin (Dox), apoptosis was enhanced by knockdown of NAP1L1, whereas NAP1L4 knockdown was resistant to apoptosis. Because acetylation status of p53 is known to regulate cell cycle or apoptosis, we next analyzed the acetylation sites of p53 under conditions of NAP1Ls knockdown. We found that knockdown of NAP1L1 increases the p53 acetylation at K382 whereas NAP1L4 knockdown augments the acetylation at K320. These results indicate that NAP1Ls regulate cell cycle and apoptosis via differential control of p53 acetylation sites.

(COI: No)

P1-135

Effects of UVA LED light irradiation on cultured RAW264.7 cells

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We studied effects of ultraviolet A (UVA) irradiation using light-emitting diode on cell growth of cultured RAW 264.7 cells. Cells were plated on 96 well plates at a density of 10^{5-6} cells/ml. After 24 hr, these cells were irradiated for varied time and maintained for 0-72 hr at 37℃. Cell images were taken by a microscope every 24 hr for 72 hr. Irradiation (365 nm) for more than 2 min suppressed cell growth. Lactate dehydrogenase release into medium was found to increase significantly at more than 3 min of irradiation, and the addition of N-acetyl cysteine as a scavenger suppressed the increase caused by the 5 min-irradiation. Formation of 8-hydroxy-2'-deoxyguanosine and malondialdehyde were also increased by the 5 min-irradiation. Finally, to detect reactive oxygen species induced in the medium irradiated by the light, we measured EPR(electron paramagnetic resonance) signals in the presence of spin trapping agents (TPC and DMPO) by EPR spectrometer. NaN_3 decreased the spin peaks formed by TPC and histidine decrease the peaks by DMPO. These measurements indicate that singlet oxygen ($^1O_2$) is initially induced and the singlet oxygen is converted into hydroxyl radical. These results suggest that ROS induced by UVA irradiation increase membrane permeability, DNA damage and lipid peroxidation, and results in cell growth inhibition.

(COI: No)

P1-136

What happens when cell proliferation is inhibited by Cs?

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Cesium (Cs) is one of the alkali metal elements as well as potassium (K) and sodium. Distribution of Cs in the whole bodies had been investigated. However, it is not clear that how the Cs is transported through which kinds of way at the molecular levels, and that the effect of Cs on the cell metabolisms. We reported that the proliferation of HeLa cells inhibited by Cs but not other alkali metals. The proliferation decrease is dependent on Cs concentration. The cell viability was assessed by two different methods, i.e., LDH assay and flow cytometric assay, and the cell membrane was not damaged by 10 mM Cs treatment. K^+ channel blocker also inhibited cell growth. Reversible cell-cycle arrest occurs by inhibition of ATP-sensitive K^+ channels. Quinidine treatment showed cell-cycle G0/G1 arrest and inhibition of proliferation, and how was Cs treatment. Cell-cycle was evaluated by using HeLa cell with fucci-system established by Miyawaki. Quinidien treatment cell showed G0/G1 arrest but Cs treatment cell did not show the G0/G1 arrest. Cs-treated cell seemed the same as control cell-cycle. The results suggested that effect of Cs on cell proliferation was not inhibited effect but suppression effect.

(COI: No)

P1-137

D-allose inhibits cancer cell growth by an induction of thioredoxin interacting protein (TXNIP) and inhibition of Glut1 expression

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Glucose transporters are members of membrane proteins that facilitate glucose transport. Of those, glucose transporter 1 (Glut1) is responsible for the basal glucose uptake and highly expressed in erythrocytes and endothelial cells. Interestingly, Glut1 is over-expressed in many cancer cells and is play an important roles on the cell growth. Rare sugar D-allose dose-dependently induced TXNIP expression and inhibited the Glut1 expression in HuH-7, MB231 and SH-SY5Y cells. And the glucose uptake in HuH-7 cells was significantly inhibited by D-allose treatment. Both the TXNIP over-expression and D-allose treatment inhibited hypoxia-inducible factor-1 alpha (HIF-1 α) expression, that is the transcription factor of Glut-1 and is over-expressed in many cancer cells, resulting the reduction of Glut1 expression. Thioredoxin is known to increase the promoter activity of HIF-1 α via the nuclear factor-kappa B (NF-κB), p50-RelA subunits binding. As TXNIP inhibits the thioredoxin activity, over-expression of TXNIP or D-allose treatment decreased the promoter activity of nuclear factor-kappa B and decreased the HIF-1 α expression. Along with the previously reported mechanism of D-allose that inhibits the cancer cell growth by stabilizing the cell cycle inhibitor p27 protein and inducing G1 cell cycle arrest, this study revealed a novel mechanism of the cancer cell growth inhibition by D-allose via a reduction of Glut1 expression.

(COI: No)

P1-138

Anticancer Activity of Isoamericanol from Jatropha curcas Extacts on the Human Breast Cancer Cell, MCF-7

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Various parts of Jatropha curcas trees have long been used as traditional medicine in African and Asian countries for a variety of sicknesses. There are some reports that show anticancer activity in vitro by the applications of J. curcas seed extract. J. curcas seed extract is a source of oil for biodiesel energy, but it involves a great amount of seed waste. The collaborative research with Kagawa University, Japan and Chiang Mai University, Thailand has succeeded in extracting the organic and aqueous layers with both ethyl acetate (EtOAc) and methanol (MeOH) from the waste. Furthermore, crystallization of isoamericanol from the organic layer of the MeOH extracts has been achieved. In our previous study, isoamericanol was shown to have high antioxidative activity, yet the anticancer activity of isoamericanol has never been reported. In this study, the anticancer activity of isoamericanol is tested on the human breast cancer cell, MCF-7. The inhibition of MCF-7 cell growth by isoamericanol is dose-dependent (25, 50, 100 μg/ml). We further examine the effect of isoamericanol on the cell cycle and apoptosis. Microarray analysis is also performed to identify new possible molecular pathways for anticancer therapies with isoamericanol.

(COI: No)

P1-139

Involvement of D-allose-inducible tumor suppressive factor TXNIP (thioredoxin interacting protein) *in vivo* tumor model

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D-allose, the C3-epimer of D-glucose, has an anti-proliferative effect on various cancer cell lines. We have reported that D-allose treatment caused up-regulation of thioredoxin interacting protein (TXNIP), an anti-tumor protein down-regulated in cancer cells. The anti-proliferative effect of D-allose is due to the up-regulation of TXNIP which causes cell cycle arrest at the G1/S checkpoint. Here we analyzed the signaling mechanisms of TXNIP up-regulation caused by D-allose in the hepatocarcinoma cell line HuH-7. The results suggest that both p44/p42 MAPK pathway and p38MAPK pathway participate in the TXNIP up-regulation. We further analyzed downstream molecules responsible for the TXNIP up-regulation. Moreover, we performed*in vivo* administration analysis of D-allose. The oral squamous carcinoma HSC-3 cells were used in a xenograft model with nude mice. The results show that D-allose exerts growth inhibitory effects on cancer tissues, and that TXNIP up-regulation is possibly responsible for this effect. Overall, present works would make a great contribution to the establishment of a new strategy of cancer therapy utilizing D-allose and TXNIP.

(COI: No)

P1-140

Coagulation factor IX regulates cell migration and adhesion in vitro

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Objective: Coagulation factor IX (F9) is thought to circulate in the blood as an inactive zymogen before being activated in the coagulation process. The effect of F9 on cells is poorly understood. This study aimed to evaluate the effects of intact F9 and its cleavage fragments on cell behavior.
Methods: A431 cells (derived from human squamous cell carcinoma), Pro5 cells (derived from mouse embryonic endothelial cells), Cos7 cells, and human umbilical vein endothelial cells were utilized in this study. The effects of F9 and its cleavage fragments on cell behavior were investigated in several types of experiments, including wound-healing assays and modified Boyden chamber assays.
Results: The effect of F9 depended on its processing; full-length F9 suppressed cell migration, increased adhesion to matrix, and enhanced intercellular adhesion. In contrast, activated F9 enhanced cell migration, suppressed adhesion to matrix, and inhibited intercellular adhesion. An activation peptide that is removed during the coagulation process was found to be responsible for the activity of full-length F9, and the activity of activated F9 was localized to an EGF domain of the F9 light chain.
Conclusion: Full-length F9 has a sedative effect on cells, which is counteracted by activated F9 in vitro. Thus, F9 may play roles before, during, and after the coagulation process.

(COI: No)

P1-141

Transient receptor potential cation channel 3 (TRPC3) regulates proliferation and migration via phosphorylation of STAT5 in human melanoma

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Background: It is well known that melanoma has a poor prognosis due to its rapid progression and high metastatic ability. TRPC are activated by changes of temperature or membrane voltage, resulting in activation of intracellular responses. Here, we investigate whether TRPC3 regulates cell proliferation and migration of human melanoma.
Material: C8161 cells, a BRAF wild type human melanoma cell line, were used in this study. In order to examine the role of TRPC3, lentivirus shRNA encoding either TRPC3 or scramble was used.
Result: mRNA and protein of TRPC3 were expressed in multiple human melanoma cell lines. Knockdown of TRPC3 in C8161 cells inhibited proliferation ($p<0.0001$). Pyr3, a pyrazole compound which is known to inhibit selectively TRPC3, suppressed cell proliferation (IC_{50} 12.99 μM). Both knockdown of TRPC3 and Pyr3 decreased path length of migration ($p<0.01$, $p<0.01$ respectively). Pyr3 also inhibited phosphorylation of signal transducer and activators of transcription (STAT) 5, suggesting that TRPC3-induced proliferation and migration were regulated by, at least in part, the JAK/STAT signaling pathway.
Conclusion: Inhibition of TRPC3 suppressed cell proliferation and migration, suggesting that TRPC3 could be a novel target for treating human melanoma.

(COI: No)

P1-142

The PKA- and p38-mediated phosphorylation processes are involved in the stimulatory effect of TNF-α on K^+ channel activity in human proximal tubule cells

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We previously reported that a proinflammatory cytokine, TNF-α, stimulated activity of an inwardly rectifying K^+ channel in cultured human proximal tubule cells. We also found that the stimulatory effect of TNF-α on K^+ channel activity partly contributed to the cytotoxicity of this cytokine. In this study, we investigated the mechanisms of action of TNF-α on K^+ channel activity, using the patch-clamp technique. In cell-attached patches, TNF-α (20 ng/ml) increased K^+ channel activity in a few minutes, which was blocked by an analog of the soluble TNF receptor, etanercept (10 μg/ml). Since the activity of this K^+ channel was stimulated by PKA- or PKG-mediated phosphorylation, we tested inhibitors of these protein kinases. A PKA-specific inhibitor, KT5720 (500 nM), but not a PKG-specific one, KT5823 (1 μM), blocked the effect of TNF-α. Furthermore, a specific inhibitor of p38 MAPK, SB203580 (1 μM), also blocked the TNF-α-induced activation of channel, whereas an ERK inhibitor, U0126, (20 μM) or a Jnk inhibitor, SP600125 (10 μM), failed to block it. A membrane-permeant cAMP analog, 8Br-cAMP (100 μM), stimulated channel activity in the presence of SB203580. These results suggested that the stimulatory effect of TNF-α K^+ channel activity in cultured human proximal tubule cells was receptor specific and dependent at least in part on the PKA- and p38-mediated phosphorylation processes.

(COI: No)

P1-143

Calcineurin B homologous protein 3 (CHP3) regulates phosphorylation of GSK3β

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Calcineurin B homologous protein3 (CHP3) is a EF-hand calcium-binding protein mainly expressed in heart, but its function remains largely unknown. We used adenoviral-based RNA interference system to knock down CHP3 expression in rat neonatal ventricular cardiomyocytes. Knockdown of CHP3 result in significant enlargement of cardiomyocyte size and increase the protein expression level of the pathological hypertrophy marker ANP. Furthermore, the phosphorylation level of GSK3beta was dramatically elevated. On the contrary, CHP3 overexpression results in increment of GSK3beta phosphorylation induced by insulin stimulus. Co-immunoprecipitation experiments demonstrated the interaction of CHP3 with GSK3beta. These results suggest that CHP3 serves as a novel regulatory factor for GSK3beta, which modulates cardiomyocyte hypertrophy.

(COI: No)

P1-144

Oxidized S100A4 inhibits the activation of protein phosphatase 5 through S100A1 in MKN-45 gastric carcinoma cells

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S100 proteins bind to numerous target proteins, as well as other S100 proteins and activate signaling cascades. S100 proteins can be modified by various post-translational modifications, such as phosphorylation, methylation and acetylation. In addition, oxidation is important for modulating their activities. Previous studies have shown that S100A1 interacts with S100A4 in vitro and in vivo. Due to this potential crosstalk among the S100 proteins, the aim of the present study was to examine whether S100A4 modulates the activity of S100A1. S100A4 was readily oxidized and formed disulfide-linked dimers and oligomers. Although non-oxidized S100A4 bound to protein phosphatase 5 (PP5), the Cu-oxidized S100A4 failed to bind PP5. Instead, the Cu-oxidized S100A4 directly interacted with S100A1 and prevented PP5 activation. Hydrogen peroxide induced S100A4 oxidation in MKN-45 gastric adenocarcinoma cells and decreased S100A1-PP5 interaction, resulted in the inhibition of PP5 activation by S100A1. These data indicate that oxidized S100A4 regulates PP5 activity in a unique manner under oxidative stress conditions.

(COI: No)

P1-145

Knockdown of DEAD-box protein 5 (DDX5) represses NF-kB transcriptional activation

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DEAD-box protein 5 (DDX5) is one of the DEAD-Box families that has a helicase activity. Recent studies suggest that DDX5 also serves as a regulator of transcription factors. Diacylglycerol kinase (DGK) converts diacylglycerol to phosphatidic acid in phosphoinositide turnover. We identified DDX5 as a new binding partner of zeta type DGK (DGK ζ). Transcription factor nuclear factor-kappa B (NF-kB) is known to play a crucial role in various processes, such as immune response, inflammation, cell proliferation, and oncogenesis. We previously reported that DGK ζ knockdown facilitates inhibitor of kappa B (IkB) degradation and phosphorylation of NF-kB p65 subunit, thereby upregulating of NF-kB transcriptional activity. In this study, we examined how DDX5 affects NF-kB pathway. To this end, we knocked down DDX5 in HeLa cells and performed immunoblot analysis and luciferase assay. We found that in these cells phosphorylation levels at Ser536, Ser468, and Ser311 of NF-kB p65 subunit were attenuated compared with wild-type cells. On the other hand, IkB level remained unchanged. Luciferase assay revealed that DDX5 knockdown represses NF-kB transcriptional activity. Collectively, these results suggest that DDX5 knockdown has no effect on IkB degradation, but attenuates phosphorylation of the p65 subunit, thereby downregulating NF-kB transcriptional activity.

(COI: No)

P1-146

Role of Steroidogenic acute regulatory protein-related lipid transfer domain containing 10 (STARD10) in hepatic inflammation

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Steroidogenic acute regulatory protein related lipid transfer (START) domain containing 10 (STARD10) is a member of the START domain containing lipid transfer protein family. We have previously shown that STARD10 is highly expressed in the liver and involved in regulating expression of PPAR α -target genes. Since the activation of PPAR α negatively regulates NF-κ B activity that promotes inflammatory gene expression, STARD10 may exert anti-inflammation activity. The aim of this study was to clarify the role of STARD10 in inflammation in the liver. We examined the effect of STARD10 using *Stard10* knockout (*Stard10*$^{-/-}$) mice. *Stard10*$^{-/-}$ mice fed with high fat diet gained weight in a manner similar to WT mice. However, the liver of *Stard10*$^{-/-}$ mice was smaller in size and accumulated significantly less cholesterol and triglyceride than that of WT mice. Sizes of individual lipid droplet of hepatocytes of *Stard10*$^{-/-}$ mice was significantly smaller than those of WT mice. These results are consistent with the down-regulation of PPAR α target gene such as *Mogat*, which is involved in triglyceride synthesis, was down-regulated in *Stard10*$^{-/-}$ mice. Gene expression levels of IL-1 β and TNF- α were increased in *Stard10*$^{-/-}$ mice, suggesting that STARD10 regulates these genes through inhibition of NF- κ B activity. These results indicate that STARD10 is involved in the regulation of lipid storage and inflammatory responses in the liver through PPAR α -dependent mechanism.

(COI: No)

P1-147

Analysis of interdomain interactions in PLCζ by the combined expression of split mutants

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Phospholipase C ζ (PLC ζ) is a strong candidate for mammalian sperm-derived factor that triggers Ca^{2+} oscillations required for the egg activation at fertilization. The PLC ζ protein consists of EF-hand domain in the N-terminus, X and Y catalytic domains, and C-terminal C2 domain. Although the three-dimensional structure obtained by computer modeling predicts the contacts between domains at EF/C2, X/Y, and Y/C2 interfaces, the functional significance of these putative interactions for the catalytic activity has yet to be elucidated. In the present study, we constructed several truncated mutants of human PLC ζ by splitting at the linkers between two flanking domains, and evaluated the Ca^{2+} oscillation-inducing activity by injecting their cRNAs into mouse eggs. While none of the mutants examined induced Ca^{2+} oscillations on its own even at the high level of expression, the pairwise expression of complementary split mutants, such as EF-X and Y-C2, or EF-X-Y and C2, generated the normal pattern of Ca^{2+} oscillations, suggesting the combinatorial contribution of the interdomain interactions among four domains, for PLC ζ to adopt the active conformation. It was also shown that the linker region between X and Y domains is not necessary for the PLC ζ activity, since the pair of EF-X and Y-C2 mutants lacking XY linker could, if less effectively, induced Ca^{2+} oscillations. Results of experiments for other combinations of split mutants, as well as for some circularly permutated mutants, will also be presented to discuss the structural requirements for the catalytic activity of PLC.

(COI: No)

P1-148

FABP7 is involved in epigenetic modification of mouse caveolin-1 gene promoter

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Introduction: Intracellular lipid dynamics are closely associated with the epigenetic status such as DNA methylation and histone modification. Fatty acid-binding protein 7 (FABP7), which binds to PUFAs, is expressed by astrocytes in developing brain. We have so far shown that FABP7 is involved in the lipid raft function in the astrocytes through its gene regulation of caveolin-1, a scaffold protein of lipid raft. In this study, we sought to examine whether the epigenetic modification of caveolin-1 gene promoter was dependent on FABP7 levels.

Methods and Results: Immunostaining analysis showed that FABP7 was localized in both cytosol and nucleus in the mouse astrocytes. In qPCR, caveolin-1 gene (Cav-1) expression was decreased in FABP7-KO astrocytes compared with wild-type (WT) astrocytes. Luciferase reporter assay using FABP7-transfected-NIH-3T3 cells revealed that the activation of an approximately 200bp upstream region from Cav-1 transcriptional start codon was dependent on the FABP7 levels. Furthermore, CHIP assay revealed that the level of H3K27 acetylation in Cav-1 promoter was increased by FABP7-transfection in NIH-3T3 cells. In bisulfite sequencing analysis, FABP7-KO astrocytes contained significantly higher methylated CpG sites in Cav-1 promoter than WT.

Discussion: FABP7 may have the role in the regulation of histone acetylation and DNA methylation possibly through its effect on the cellular lipid metabolism.

(COI: No)

P1-149

The difference of properties of hypotonic swelling among different cell species

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We already reported that the rate of maximum swelling after hypotonic challenge differed among different cell species. For example, HeLa cells showed cell swelling 1.6 times higher than isotonic condition by hypotonic challenge. SH-SY5Y cells showed cell swelling only 1.1 times higher than control condition. We focused on the difference of hypotonic swelling between HeLa cells and SH-SY5Y cells. Both HeLa cells and SH-SY5Y cells are suspended by trypsin treatment and cell volume was measured by flowcytometry. HeLa cells showed rapid increase of cell volume after hypotonic challenge. This hypotonic swelling was suppressed by bumetanide, NKCC blocker. Isotonic cell volume did not changed by bumetanide. SH-SY5Y cells showed little swelling by hypotonic challenge as reported last year. SH-SY5Y cells showed cell shrinkage by applying bumetanide in isotonic condition. By applying hypotonic solution with bumetanide SH-SY5Y cells showed cell swelling which is almost same absolute value as the hypotonic challenge without bumetanide. We found that SH-SY5Y cells showed apparent cell swelling like HeLa cells if we plotted the ratio of cell volume with bumetanide. We also examined the expression of NKCC by RT-PCR in HeLa cells and SH-SY5Y cells. We found that both cell lines showed NKCC expression and the signal of SH-SY5Y was higher than HeLa cells. These data suggest that in SH-SY5Y cells NKCC might transport Na^+, K^+, Cl^- together with water into the cells even in isotonic condition and cell volume reached maximum value with small increase by hypotonic challenge.

(COI: No)

P1-150

The multifunctional anion transporter SLC26 gene family as potential candidate for oxyanion transport

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Toxic anions have attracted great interest in academia and industry during the last decade. However, regarding the toxic anion pathway inside the human body is poorly understood. The SLC26 gene family is a multifunctional anion transporter gene family that possesses a wide variety of anion transporting properties. Which makes it a potential candidate as a toxic anion transporter. This research involves SLC26 gene family localization and in vitro assessment of Na_3VO_4 and Na_2CrO_4 toxicity in human cells. Human cells (HeLa, HEK293, CACO2, and SH-SY5Y) were incubated for 3 days with Na_3VO_4 and Na_2CrO_4 concentration of 300 μM, 30 μM, 3 μM, 100 μM, 10 μM, and 1 μM respectively. Results show that there is a specific pattern for the SLC26 gene family localization and expression levels in human cells. Which leads to a possibility of different toxicity levels of Na_3VO_4 and Na_2CrO_4 against different human cells. These findings could be useful information to predict the toxic anion pathway inside human cells.

(COI: No)

P1-151

Possible Roles of sodium ion / proton exchanger 1 (NHE1) on the conversion of latent form of TGFβ to active form

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We already have the implications for participation of TGF β in glioma invasions toward non-cancerous brain parenchyma, and premetastatic niche formations on lymph node metastases of head and neck squamous cell carcinomas. Since upregulations of mRNA or TGF β precursor protein were observed in either cases, we expect them as at least a part of mechanisms for progression of the malignancy or as a possible target for anti-metastasis therapy. Importantly, TGF β is secreted in inactive form, namely latent from. Therefore, measurement of TGF β activities are indispensable to assess whether the factor really functions or not, and the mechanisms convert TGF β from latent to active has physiological as well as pathophysiological significance. NHE1 acts as an ion transporter excretes proton toward extracellular space owing to the concentration gradient of sodium ion as a driving force. Simultaneously, NHE1 also acts as an anchor of actin-cytoskeleton on plasma membrane via its intracellular domain. We have also found aberrant overexpression of NHE1 in gliomas as well as squamous cell carcinomas, and furthermore, observed decreases of TGF β activities in conditioned culture media prepared from NHE1 knockdown cells or NHE1 inhibitor treated cells in preliminary experiments. We would like to discuss as to how the participations of NHE1 in TGF β conversion is possible.

(COI: No)

P1-152

Evaluation of anti-angiogenic drug for endothelial cells derived from glioma stem cells

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Central nervous system (CNS) is mainly composed of neuron, astrocyte and oligodendrocyte. They are derived from neural stem cells (NSCs), which have the characteristics of self-renewal and multipotency. In glioblastoma (GBM), which is the most malignant brain tumor, the existence of glioma stem cells (GSCs) in GBM is reported in 2004. GSCs have similar features with NSC in terms of self-renewal and multipotency. GSCs also have high tumorigenesis and therapeutic resistance. GBM is one of the vascular rich tumors, and neovascularization is normally performed by recruiting endothelial cells from brain vessels. Vascular endothelial growth factor (VEGF) is a critical regulator of this process. In addition, recent study says that GSC can transdifferentiate into endothelial cells and vascular pericytes to support tumor environment. In our research, 15 percent of tumor vessels include the GSC derived endothelial cells, but the mechanism and features are little known. Here, we report two kinds of tumor angiogenesis from the point of VEGF pathway. And focusing on the effect of anti-angiogenic drugs for these two angiogenesis, we suggest new therapeutic target leads to development of new anti-angiogenic drugs.

(COI: No)

P1-153

Identification of amino acid residues involved in the TRPA1 inhibition by utilizing species specific differences

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Pain is a harmful sensation that usually arises from noxious stimuli. Transient Receptor Potential Ankyrin 1 (TRPA1), a member of TRP subfamilies, is one of those targets for studying the pain mechanism. TRPA1, a sole member of TRPA subfamily is known to be activated by various stimuli such as noxious cold (potentially in rodents), pungent natural products (like cinnamaldehyde; CA) and environmental irritants (like acrolein). Since TRPA1 is an attractive target for pain therapy, many TRPA1 antagonists have been developed and some of them function as analgesic agents. Here, I show that HC-030031 (HC), one of the most potent mammalian TRPA1 antagonists, did not inhibit heterologously expressed western clawed frog TRPA1 (fTRPA1). In a heterologous expression system with *Xenopus* oocytes, HC failed to inhibit fTRPA1 activation elicited by CA (a TRPA1 agonist) but inhibited CA-evoked currents of human TRPA1 (hTRPA1) with a dose-dependent manner. Chimeric studies between fTRPA1 and hTRPA1 as well as point mutant channel analyses revealed that one specific amino acid residue located within the transmembrane domain was partially involved in the inhibitory action of HC. These findings are based on species differences in sensitivity of TRPA1 antagonists and provide novel insights for the structural-function relationship of TRPA1.

(COI: No)

P1-154

Visualization of fluctuating motions of the selectivity filter in the potassium channel: A computational study

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Ion channels are membrane proteins that allow ions to permeate through them and generate electrical signals. Since the determination of the x-ray crystal structure of the KcsA potassium channel, ion permeation through the K^+ channel have been visualized by computer simulation using the molecular dynamics (MD) method. Simulations revealed the motions of ions in the selectivity filter (SF), the most constricted part of the channel, connecting the extracellular solution and the wide cavity being located on the intracellular side. Partially dehydrated ions in SF are coordinated by the carbonyl backbone of SF. The electrical interaction between the ions and the backbone is so strong that it could alter the conformation of the backbone, however, the extent of its change is not known. Here, we performed the MD simulation of the outward current through the Kv1.2 channel and observed the fluctuation of the channel. The fluctuation of the backbone was found to be suppressed due to the interaction with the permeating ions. There are four threonines in SF, whose hydroxyl groups usually fluctuate vigorously in the cavity. When an ion enters into the cavity and comes close to SF, the ion interacts with one of them first, and then coordinates to all four sidechains to make a stable complex. An analysis of the relation between the fluctuations of hydroxyl groups and the current shows that there exists a moderate correlation between them. Thus, the current measured by the single channel recordings reflects the fluctuation of the channel to some extent.

(COI: No)

P1-155

Functional expression of $P2X_7$ receptors in odontoblasts

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Extracellular purine nucleotides activate receptors of the P2 receptor family that are subdivided into two structurally distinct subfamilies: the inotropic P2X and G-protein coupled P2Y receptors. Although immunohistochemical expression of the P2X receptor subtypes-$P2X_2$, $P2X_4$, $P2X_6$, and $P2X_7$-in odontoblasts has been reported, their physiological and detailed pharmacological properties remain unclear. We thus examined the functional expression of $P2X_7$ receptors in mouse odontoblasts. Currents induced by $P2X_7$ receptor activation were recorded by whole-cell patch-clamp recording with a holding potential of -70 mV. Extracellular application of adenosine 5'-triphosphate dipotassium salt (K^+-ATP), a nonselective agonist for P2 receptors, evoked inward currents with an amplitude of -2.9 ± 0.6 nA (n = 3). These currents showed a significant desensitizing effect by repetitive application of K^+-ATP. Extracellular application of 300 μM 2'(3')-O-(4-benzoylbenzoyl)adenosine 5'-triphosphate triethylammonium salt (BzATP), a selective $P2X_7$ agonist, also evoked inward currents with an amplitude of -2.8 ± 0.8 nA (n = 6). KN-62 (10nM), a selective $P2X_7$ antagonist, significantly suppressed the BzATP-induced inward currents to 81.9 ± 9.7 % (n = 6). In addition, application of 300 μM BzATP induced a positive shift in the reversal potential. The estimated permeability of BzATP-induced currents was 11.6, compared with that without BzATP (1.0). These results indicate that odontoblasts express $P2X_7$ receptors.

(COI: No)

P1-156

Modeling effect of age-related changes in ionic systems on action potential of pulmonary vein myocardium

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The pulmonary vein contains a myocardial layer that is capable of generating spontaneous or triggered action potentials, which is considered to play a central role in the generation and maintenance of atrial fibrillation. The pulmonary vein myocardial layer is extending from the left atrium, but has less negative resting membrane potential due to a lower density of the inwardly rectifying K^+ current. Although electrophysiological and pharmacological characteristics of the pulmonary vein myocardium are reported in various literatures, a comprehensive understanding of the spontaneous action potentials generated in the myocardial layer is yet to be assessed. Here, we integrated electrophysiological properties of the pulmonary vein myocardial layer on the basis of the Kyoto model. Based on the preceding research which reported that approximately half of the isolated pulmonary vein myocardial layer exhibited spontaneous action potential and the remaining half were quiescent, we constructed various combinations of the pulmonary vein myocardial models in order to represent the variation of the action potentials. On the basis of the transcriptome data from young and aged myocardial tissues, we expanded the combinations to represent the "aged" pulmonary vein myocardial layer. As a result, we predicted that the spontaneous action potentials, including burst-like action potentials, are more likely to be observed in "aged" combinations than "young" combinations.

(COI: No)

P1-157

Water flow in mantle cavity of bivalve observed by high-field MRI

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Water flow in the mantle cavity of bivalves (*Mytilus galloprovincialis*) was measured by phase-contrast magnetic resonance imaging (PC-MRI), and transient changes in water velocity were imaged by using the inflow effect of T_1-weighted MRI. All experiments were done by 7 T high-field MRI. During steady ventilation, water velocity in the inhalant aperture, lower mantle cavity, interlamellar space and the exhalant aperture were 40-20 mm s^{-1}, 10-20 mm s^{-1}, 5-10 mm s^{-1} and 50 mm s^{-1}, respectively. Spontaneous opening of the shells caused a quick increase of the flow in the mantle cavity within 1 min. A high correlation was detected between the area in the inhalant aperture and that in the exhalant siphon. However, the flow in the interlamellar cavity showed a low correlation with that in the inhalant aperture. The flow in the right and left interlamellar cavities changed independently. These results suggested that the mussel could control flow in a local area of the gill by changing activities lateral cilia in the demibranches. In conclusion, a combination of PC-MRI and the inflow effect of T_{1w}-MRI allowed us to perform quantitative flow analysis in all of the cavities in the mussel.

(COI: No)

P1-158

Analysis of Atg9-containing membrane structures: ultrasturecure and function in autophagy

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Atg9 is a multispanning membrane protein that is conserved from yeast to human. Atg9 is essential for autophagy and considered to be directly involved in the early step of autophagosome formation. We have previously reported that yeast Atg9 is localized on the cytoplasmic small vesicle, an Atg9 vesicle. Atg9 vesicles are derived from the Golgi apparatus and transport vesicle- tethering proteins to the autophagosome formation site. In mammalian cells, Atg9 was reported to be localized to the trans-Golgi network, late endosomes, and recycling endosomes. In order to characterize the mammalian Atg9-containing membranes, we examined the localization of Atg9 in HEK293 cells that stably express Atg9-GFP. Atg9-GFP was partially localized to static membrane structures, which are colocalized with a recycling endosome protein, transferrin receptor. Atg9-GFP was also observed as multiple puncta that move rapidly throughout the cytoplasm. We succeeded in isolation of these cytoplasmic small membranes. The immunoprecipitated membranes did not contain common organelle marker proteins. Electron microscopic analysis revealed that these are small vesicles resembling the yeast Atg9 vesicle. These results suggested that Atg9-specific membrane structures also exist in mammalian cells. Proteomic analysis of these membranes will lead to a better understanding of the function of Atg9 vesicles.

(COI: No)

P1-159

AQP11 null mice enhance autophagy activity in the proximal tubule before polycystic kidney disease

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Water channel AQP11 is expressed in the various tissues. In the kidney, AQP11 is selectively expressed in the proximal tubule. AQP11 null mice are dead from polycystic kidney disease within two months after birth. The intracellular vacuoles and the cysts were reported to be caused by ER stress as documented by the microarray data of the kidneys from P7 mice. The alternation of gene expression that related autophagy was also performed by our microarray in the kidney from P3 and P28. Based on these results, we initiated the study on autophagy in the kidney of AQP11 null mice. We generated GFP - LC3 (microtubule associated protein light chain 3) transgenic mice in the background of AQP11 null to visualize and monitor the activity of autophagy. Specifically, the expression of GFP fluorescence was analyzed in the frozen sections of the kidney. The primary cultured cells from the proximal tubule were also employed to quantify the autophagy activity. The expression of GFP-LC3 was increased in the vacuolated proximal tubule of P7 AQP11 null mice, which become more intense at P14 when the cysts were formed. The autophagy was absence in the other segments of the nephron. The primary cultured cells from the proximal tubules as proved by AQP11 expression revealed that the number of GFP fluorescence was 2 fold more in AQP11 null than that of wild type, although there were no morphological and viability differences. Our results suggested that autophagy may play an important role for the survival of vacuolated cells and later cyst formation in AQP11 null mice.

(COI: No)

P1-160

Analysis of accumulated proteins in abnormal lysosomes of brain tissue in cathepsin D-deficient mice

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Cathepsin D, a major lysosomal aspartic proteinase, is ubiquitously expressed. Cathepsin D knockout (CD-/-) mice die at ca.26 days after birth with massive neurodegeneration, intestinal necrosis, and lymphopenia. In neurons of CD-/- mice, abnormal lysosomes with typical hallmarks for neuronal ceroid lipofuscinosis, autophagosomes and autolysosomes accumulate in neurons. We previously found that in such lysosomes subunit c of mitochondrial ATP synthase accumulates, which is one of substrates of CD. However, there are many other candidates for CD substrates in the brain. In the present study to comprehensively identify proteins accumulating in the lysosomes of CD-/- mice, we modified the subcellular fractionation of lysosomes from mouse brains and investigated the comportments of the lysosomes of CD-/- mice through mass spectrometry analysis.

(COI: No)

P1-161

Membrane dynamics in yeast lipophagy

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Triglycerides and sterol esters stored in lipid droplets (LDs) are used for energy production but they are also utilized to generate membranes and signaling mediators. Due to the physiological importance of these processes, how lipid esters in LDs are mobilized has been a focus of intensive studies. A large portion of lipid esters are hydrolyzed by cytosolic enzymes, but it is becoming clear that an autophagic process called lipophagy is also involved in degrading LD-laden lipid esters. However, details of lipophagy are not characterized yet.

In the present study, in order to understand how lipophagy is executed, we examined the membrane dynamics of the yeast lipophagic process by utilizing quick-freezing and freeze-fracture replica labeling electron microscopy (QF-FRL). By taking advantage of the merit of QF-FRL, which can define distribution of membrane molecules, both proteins and lipids, at the nanoscale, we could observe that distribution of phospholipids changes significantly in relation to LDs, the vacuole (lysosome), and autophagic bodies (i.e., structures in the vacuolar lumen). Implication of the observation with regards to the mechanism of lipophagy will be discussed.

(COI: No)

P1-162

Biogenesis of nuclear lipid droplets

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The lipid droplet (LD) is thought to be formed at the endoplasmic reticulum (ER), and is composed of a core of neutral lipids and a phospholipids monolayer. So far LD has been considered a cytoplasmic organelle that plays different physiological roles including as a platform of protein degradation for example. However, LDs are also observed in the nucleus of certain cell types such as hepatoma cells. In the present study, we addressed the mechanism that nuclear LDs (nLD) are generated. nLDs were devoid of perilipin-2 and virtually absent in adipocytes and steroidogenic cells although abundant cytoplasmic LDs (cLD) are present, indicating that nLDs and cLDs form differently. Interestingly, nLDs were associated with polymyelocytic leukemia (PML)-nuclear bodies (NB) and with intranuclear membranes extending from the nuclear envelope. The nLD-PML body complex was labeled for ubiquitin, SUMO1 and p53, suggesting that nLDs function as a site of protein modification and transcription control. The number of nLDs was reduced by knocking down PML isoform-II (PML-II), whereas overexpression of PML-II enhanced the nLD formation, probably by increasing the intranuclear membrane that harbors lipid-ester synthesizing enzymes. These results indicated that nLDs is a unique structure correlated with the PML-NB function.

(COI: No)

P1-163

Subcellular distribution of PI(3, 5)P_2 revealed by quick-freezing and freeze-fracture replica labeling

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Our knowledge about the lipid distribution is limited because of the difficulty in visualizing lipids. To overcome this, we have developed a unique, electron-microscopic method, named quick-freezing and freeze-fracture replica labeling. This method physically fixes membrane lipids and enables to determine their precise localization.
In this study, we applied the method to phosphatidylinositol-3, 5-bisphosphate (PI(3, 5)P_2), which is important for the endolysosomal function and implied in the pathogenesis of motor neuron diseases. We utilized recombinant ATG18p from *S. cerevisiae* as a probe and blocked its binding to PI3P in the presence of an excess amount of the $p40^{phox}$ PX domain, which only binds to PI3P and not to PI(3, 5)P_2.
In *S. cerevisiae*, the PI(3, 5)P_2 labeling was observed in vacuole upon hyperosmotic shock. Interestingly, the labeling was concentrated in a vacuole domain where transmembrane proteins were excluded, whereas PI3P existed in the entire vacuole. The domains were frequently invaginated toward the lumen and coincided with membrane contact sites either between neighboring vacuoles or between the vacuole and the nucleus.
In HeLa cells, PI(3, 5)P_2 was labeled in intracellular vesicles with tubular extensions, which morphology suggests them to be endosomes. This is the first nanoscale demonstration of the PI(3, 5)P_2 distribution. Our approach should help analyze the function of PI(3, 5)P_2 in both physiological and pathological contexts.

(COI: No)

P1-164

UBXD8 deletion in hepatocytes induced more evident abnormalities in female than in male mice

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Background: Using hepatoma cell lines, we found that UBXD8 is engaged in transporting ubiquitinated ApoB from LDs to proteasomes and that knockdown of UBXD8 causes aberrant accumulation of ApoB in the ER lumen facing the LD. To address the function of UBXD8 in liver in vivo, we generated hepatocyte-specific UBXD8 knockout (U8-LKO) mouse and examined the phenotype in detail.
Method: Mice with the floxed exon1 of the *Ubxd8* locus were crossed with mice expressing the albumin promoter-driven Cre recombinase to generate U8-LKO mice. U8-LKO mice and their age-matched littermates (control) were fed either a normal diet or a high-fat diet for 26 weeks starting at 4 weeks old.
Results: (1) After 26 weeks of a normal diet feeding, histological analysis of liver sections did not reveal any difference between U8-LKO and control mice, and any sign of hepatic damage or steatosis was observed. (2) When mice were fed a high-fat diet for 26 weeks, 40% of female and 80% of male of the control mice showed microvesicular steatosis primarily in the perivenular area (zone 3), whereas 60% of female and 27% of male of the U8-LKO mice showed macrovesicular steatosis mainly in the periportal area (zone 1). (3) The serum TG level in U8-LKO mice on a high-fat diet was significantly lower than that in control mice on a high-fat diet (female: 39 vs. 59 mg/dl, male: 44 vs. 65 mg/dl).
Conclusion: Female U8-LKO mice exhibited abnormalities in more indices than male U8-LKO mice. The result showed the importance of examining mice of both sexes, especially when studying genetically engineered mouse models for the first time.

(COI: No)

P1-165

Ring-shaped Golgi apparatus observed in the epithelial cells of rat thyroid follicles

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As we previously reported, the Golgi apparatus of pituitary gonadotropes is spherical in shape, which possibly reflects the highly isotropic arrangement of microtubules from the central microtubule organizing center (MTOC) characteristically seen in a poorly polarized endocrine cell. In contrast, epithelial cells of the thyroid follicle are well polarized and could transport membrane carriers/vesicles bidirectionally toward both the apical and basal cell surfaces. In the present study, we immunocytochemically examined the overall shape of the Golgi apparatus and the intracellular organization of the microtubule network in thyroid epithelial cells as a representative polarized cell. The overall shape of the Golgi in the thyroid epithelial cells was just like as a ring located above the nucleus, of which outer and inner surfaces were cis- and trans-sides, respectively. The MTOC immunolabeled with anti-γ tubulin antibodies, was located just beneath the apical plasma membrane, around which dense network of microtubules was observed. From the apical network of microtubules, bundles of microtubules extended along the lateral cell surface toward the bottom of the cell. Some bundles of microtubules also ran through the inner area of the ring-shaped Golgi and reached the basal cytoplasm along the outer surface of the nuclear envelope. These findings suggest that the bidirectional movements of membrane carriers/vesicles along microtubules arranged parallel to the apico-basal axis possibly determine the characteristic ring-shaped Golgi apparatus in the highly polarized epithelial cells of thyroid follicles.

(COI: No)

P1-166

Effects of Brefeldin A on Localization of Alkaline Phosphatase in McA-RH 7777 Rat Hepatoma Cells

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Alkaline Phosphatase (ALP) in McA-RH 7777 (rat Hepatoma cell) translocates from Golgi area of cytoplasm to the plasma membrane at the cell borders by cell to cell contact between adjacent cells. In the present study, we investigated changes on location of ALP by Brefeldin A in culture of McA-RH 7777 cells to examine whether vesicular transport are involved with such translocation of ALP. McA-RH 7777 cells were seeded in culture slides and cultured. After synchronized, cells were cultured for several minuites α-MEM containing Brefeldin A. Afterwards, they were fixed in Zamboni solution for 10 min at room temperature and reacted with mixed solution containing anti-GM130 and anti-ALP antibodies for double staining. After cells were reacted with mixed solution containing FITC-labeled and rodamine-labeled secondary antibodies, they were examined under a confocal scanning laser microscope. In McA-RH 7777 cells cultured in α-MEM containing Brefeldin A, many granules were showing immunofluorescence for GM130 (cis-side Golgi marker) were scattered throughout the cytoplasm. Immunofluorescence for ALP was also observed on small granules scattering throughout the cytoplasm. ALP was furthermore localized in whole plasma membrane although immunofluorecence was particularly strong at the borders between adjacent cells. The present study suggests when vesicular transport is inhibited, it makes Golgi complex disassembled and scattered throughout the cytoplasm but does not subject translocation of ALP to the plasma membrane although ALP loses directivity for transferring to local plasma membrane of the cell border.

(COI: No)

P1-167

Effects of arginine methylation via PRMT1 on Golgi body

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Cumulative of reports have shown the importance of ER stress in pathology of neurodegenerative diseases, such as Alzheimer's disease, Parkinson disease, etc. These studies indicate that the cellular events in response to ER stress should relate to the pathology of neurodegenerative diseases. To this aim, we investigated the altered genes in SK-N-SH cells under ER stress and found that Protein arginine N-methyltransferase 1, PRMT1, is up-regulated in SK-N-S H cells under ER stress. Based on this result, we addressed the following is sues; 1: Can ER stress increase the protein level of PRMT1? 2: What kind of ER stress pathways is involve in the expression of PRMT1? 3: Can ER stress a ffect the localization of PRMT1? Our results elucidated that several ER stress pathways induced PRMT1 expression, some of which affected the subcellular localization of PRMT1. Next, to examine the function of PRMT1 in the ER stress response, we downregulated the expression of PRMT1 by RNAi. When we analysed the organelle localization and function in the PRMT1 knockdown cells, the localization of Golgi apparatus was altered and the induction of GRP78 by tunicamycin was severely impaired. These results suggested a novel pathway via protein methylation that mediates organelle stress to the nucleus, possibly involved in the pathogenesis of neurodegenerative diseases.

(COI: No)

P1-168

The observation of mitochondria in spermatogenesis by FIB-SEM tomography

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Sperms are motility cells with a flagellum. The structure of sperm is constructed by head, midpiece and tail. We are able to observe specialized mitochondria that are coiled around the flagellum in the midpieace. In *Drosophila*, recently study reported that the morphogenesis of mitochondria drives the elongation of the sperms. But, it is not revealed if other animals have same mechanism. The reason why the study of morphogenesis of mitochondria is not proceeding is the resolution of the optical microscopy. It is difficult for the resolution of the optical microscopy (about 200 nm) to observe the morphology of the mitochondria that size is 300-1000 nm. Recently, FIB-SEM tomography is developed to solve these problems. FIB-SEM, is the instrument combine with the scanning electron microscopy (SEM) and the focus ion beam (FIB), is able to fabricate the sample by gallium ion and observe the surface in the same chamber. In FIB-SEM tomography, we are able to observe the 3D nanoscopic structure of the internal portion by repeats of surface removals with nm order and observations of the cross-section surface. In this study, we investigated the 3D structure of mitochondria in the spermatogenesis by the FIB-SEM tomography. And result, we found mitochondria became adhering tightly to the flagellum after contact with the flagellum. This result suggests that morphogenesis of the mitochondria is carried autonomously out before cell shape change in the spermatogenesis.

(COI: No)

P1-169

Effects of estrogen on mitochondrial elongation through MIEF1 in human breast cancer cell line

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Background: Mitochondria are dynamic organelles whose morphology is controlled by balancing between fission (fragmentation) and fusion (elongation). It is reported that estrogen affects the mitochondrial morphology in breast cancer cell line. Mitochondrial elongation factor 1 (MIEF1) is a mitochondrial outer membrane protein which suppresses dynamin-related protein 1 (Drp1)-mediated fission, leading to mitochondrial elongation. However, the precise role of MIEF1 in mitochondrial elongation regulated by estrogen is still unknown. Therefore, we examined the effect of estrogen on MIEF1 expression in MCF7, human breast cancer cell line.
Materials and methods: MCF7 cells were treated with 17 β-estradiol (E2) for 12 hours. MIEF1 mRNA and protein expressions were examined by RT-PCR and western blot, respectively. Immunohistochemistry was done by using anti-MIEF1 antibody (5.2 μg/ml, Proteintech) and anti-OxPhos V antibody (4 μg/ml, Invitrogen), a mitochondrial inner membrane protein.
Results: MIEF1 mRNA and protein expressions were increased by E2 treatment. Before E2 treatment, immunohistochemical expression of MIEF1 was diffuse, round and small dotted shapes in the cytoplasm. After E2 treatment, MIEF1 expression was changed to clear, and long cluster ones.
Conclusion: These findings suggested that MIEF1 may play an important role in mitochondrial elongation depending upon estrogen treatment in breast cancer cell line.
(COI: No)

P1-170

Cobalt inhibits the movement of motile mitochondria in the axons

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Cobalt is an important element necessary to form vitamin B12 in the human body. However, an overdose of cobalt can cause neurotoxicity and the mitochondria are the main target of cobalt toxicity. In the present study, we investigated the effect of cobalt on the axonal mitochondrial dynamics in primary cultures of rat dorsal root ganglia (DRG). Mitochondria in the axons were visualized by the transfection of lentivirus vectors containing the mitochondrial-targeted DsRed2 sequence. DRG cultures at four weeks were transfected with Mito-DsRed2 and incubated another 2-3 weeks. To observe the mitochondrial dynamics, we used time-lapse imaging. 200 mitochondrial time-lapse images were taken every 6 seconds before and after cobalt chloride treatment. The exposure duration to cobalt chloride was 24 hours. The concentrations of cobalt in replacement mediums were 200 μM, 400 μM, 600 μM or 800 μM. The exposure to cobalt inhibited the movement of motile mitochondria and the effects of cobalt was prominent from 600 μM. In addition to the cobalt effect on the motile mitochondria, mitochondrial fragmentations were observed in the axons. The mechanisms of cobalt neurotoxicity have yet to be identified however, it is possible that a high concentration of cobalt has a harmful influence on mitochondrial transport in the axons.
(COI: No)

P1-171

Rupture of vesicles and nuclear membrane under various stress on cells

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It is known that many kind of stress like ionophore administration cause cell death with vesicle and nuclear membrane rupture. We previously found that ion replacement of extracellular fluid could suppress amphotericin B-induced cell death with lysosome stabilization. In this study, we examined vesicle behavior and enzyme release after various stress including ionophore, povidone-iodine, and temperature stress. With ionophore administration, nuclear membrane rupture occurred after vesicle disappearance, and lysosomal enzyme was dispersed in entire cytosol. This rupture was inhibited with proteinase inhibitors.
(COI: No)

P1-172

Continuous stress induces multiple organelle dysfunction and subsequent cell death in rat melanotroph

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Continuous stress (CS) induces cell death of pituitary melanotrophs (MT) in rat. We used a rat CS model in which rat is kept in a cage with 1.5 cm of water for 5 days, and observed the morphological characteristics during the degeneration process under electron microscopy. The degenerating MT are classified into three types by morphological characteristics; (I) dilation of ER lumen, (II) vesicle accumulation, and (III) mitochondrial clustering. In type (I), MT initially had dilated ER and swelled mitochondria and subsequently the MT showed a degenerative morphology with a brighter cytoplasm and ruptured plasma membrane. In type (II), MT initially had dark cytoplasm and normal ER, and some autophagy related structures including mitophagy were also seen. Along with an increase of the cytoplasmic electron density, these MT were filled with huge number of vesicles such as endosomes and lysosome. In type (III), MT also had dark cytoplasm and normal ER initially, but unlike type (II), contained large mitochondria, which assembled and formed a huge mitochondrial cluster. The electron density of cytoplasm gradually decreased, although the mitochondrial cluster remained. Eventually these MT were degenerated with ruptured plasma membrane. Those observations suggest that CS elicits multiple organelle dysfunctions and causes multiple cell death in MT.
(COI: No)

P1-173

Three dimensional analyses of peroxisomes by SBF-SEM: peroxisomes proliferate by budding

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Purpose: To visualize the newly formed peroxisomes in a proliferate sate in hepatocytes in three-dimensions and at the nanometer resolution, we used serial block face-scanning electron microscopy (SBF-SEM), which images resin-embedded specimen by SEM with a sequential removal of the block surface by diamond knife microtome.
Method: Rats were fed with a diet containing peroxisome proliferator di-ethylhexyl phthalate (DEHP) for 3, 5 and 20 days. Liver tissues were fixed and histochemically stained by alkaline DAB reaction for catalase, and then subjected to SEM after embedded in resin. They were observed by SBF-SEM by back-scattered electron mode.
Result: Peroxisomes were observed as electron-dense particles in each section. Treating with DEHP, their number was gradually increased until 20 days in the hepatocytes. The three dimensionally reconstructed peroxisomes showed an almost spherical in the shape, which diameters were in a range from 0.15 to 1.37 μm. Some peroxisomes had small buds, and were observed to produce new microperoxisomes (ϕ = 0.1 μm) by fission from "mother" peroxisomes (ϕ = 1 μm).
Conclusion: Using SBF-SEM combined with alkaline DAB reaction, the ultrastructures of peroxisomes were observed at nanometer resolution. The induction of peroxisome proliferation by DEHP showed an increase of the number of peroxisomes. Precise observation suggested that small peroxisomes were newly produced by budding/fission from preexisting peroxisomes, not from the endoplasmic reticulum.
(COI: No)

P1-174

Molecular mechanism for endocytosis of TASK1 channels in adrenal medullary cells and PC12 cells in response to extracellular stimuli

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TASK channels belong to a family of K2P channels, which are involved in multiple physiological functions. We have recently elucidated that activation of muscarinic receptors or a decrease in external pH in rat AM cell induces secretion of catecholamines through the inhibition of TASK1-like channels. Additionally, we indicated that TASK1 channels in rat AM and PC12 cells are translocated from the cell membrane to the cytoplasm in response to NGF and muscarine. Here, we explored the molecular mechanism for this internalization of TASK1 channels in rat AM and PC12 cells. We first examined the effects of various inhibitors on receptor endocytosis. Both NGF- and muscarine-induced internalization of TASK1 channels were remarkably suppressed by chlorpromazine, suggesting that TASK1channels were internalized in a clathrin-dependent manner. Next, we investigated this signalling mechanism. Pharmacological and biochemical studies revealed that NGF-induced endocytosis of TASK1 channels was mediated by both PLC and PI3 kinase pathways that converge on PKC with the consequent activation of Src kinase. On the other hand, the muscarine-induced endocytosis of TASK1 channels was mediated by PLC, and subsequently PKC and Src kinase. However, the PI3 kinase pathway was not involved in muscarine-induced endosytosis. These results indicated that both NGF and muscarine induces the internalization of TASK1 channels in a clathrin-dependent manner, but NGF and muscarine induce endocytosis of TASK1 channels through different signaling pathways.
(COI: No)

P1-175

Dissociation of the effect of Rho agonist on endocytosis from that on cell fusion in RAW 264.7 cells

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We have previously proposed that actin superstructure, termed the zipper-like structure promotes the generation of large osteoclast in RANKL-induced osteoclastogenesis using RAW264.7 cells. Actin cytoskeletal dynamics in osteoclasts is regulated by factors including Src, Arp2/3 and small GTPase Rho. On the other hand, phagocytic activity is reported to increase during LPS-induced osteoclast fusion. Both phagocytosis and endocytosis involve actin reorganization that shares common molecules with cytoskeletal dynamics described above. Here we examined whether endocytosis is involved in osteoclast fusion. Endocytosis was estimated by the uptake of rhodamine-dextran (MW 10,000) by RAW 264.7 cells. Mononuclear precursor cells showed higher endocytosis than the fused multinucleated cells. Mononuclear cells took up dextran beneath the plasma membrane in 5 min and transferred it around the nucleus in 15 min. Dextran taken up by multinucleated cells concentrated at the ventral plasma membrane in 16 h. Rho agonist, Rho activator II had little effects on these endocytic events. The same treatment with Rho activator II inhibited the generation of the zipper-like structure and produced smaller osteoclast-like cells. The zipper-like structure was free from endocytic vesicles. The results suggest that actin reorganization involved in osteoclast fusion is distinct from that involved in endocytosis.

(COI: No)

P1-176

Scavenger receptor-mediated gliding on the dendritic membrane of MARCO cell

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Scavenger receptors were initially identified by their ability to bind to low-density lipoprotein. MARCO is a type II transmembrane protein of the class A scavenger receptor family, and is expressed primarily on macrophages and dendritic cells. Their functions were described to be similar to those of scavenger receptor AI. However, the receptor-mediated cellular dynamics still remain unclear. In this study, using the MARCO receptor gene-transfected Chinese hamster ovary cells (MARCO cell) we investigated the role of MARCO receptors in the membrane trafficking. Unlike the ordinary CHO cells, the MARCO cells were able to exhibit a highly active movement of lamellipodia which were adhesive to some type of nanoparticles. In addition, the MARCO cells formed remarkable dendritic structures in their foot trace of migration. Some nanoparticles (such as latex beads, ZnO nanoparticles, nanodiamonds, and quantum-dots) can adhere to the dendrites. Then the dendrite carried the particles by some gliding activity to the cell body. Finally, the particles were endocytosed at the cell body or at the wavefront of lamellipodia. By immunostaining, we showed that the actin filaments and some myosin molecules are present in the dendrites but microtubules are not. Cytochalasin D clearly inhibited the gliding movement of nanoparticles along the dendrites, but colchicine did not. Our results collectively suggest that MARCO receptors capture nanoparticles, and carry them along the dendritic shaft by an actin-driven gliding mechanism.

(COI: No)

P1-177

Epidermal fatty acid binding protein (EFABP/FABP5): a potential regulator of M cell differential transcytosis

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Fatty acid binding proteins (FABPs) belong to the group of conserved multigene lipid binding protein family. Results of our previous studies implied Epidermal FABP (EFABP/FABP5) association of differential M cell transcytosis of intestinal antigens. In our 16S rRNA gene microbiome analysis, *Lactobacillus acidophilus* was revealed in testinal lumen of EFABP null mice and was not in wild type mice. Dendritic cell (DC) specific intercellular adhesion molecule 3 (ICAM3)-grabbing nonintegrin (DC-SIGN) is the major molecule to recognize*L. acidophilus*. Membranous fraction of intestinal homogenate western blot analysis showed both DC-SIGN and DC-SIGN neckless isoform expression in EFABP null mutant, while only DC-SIGN expression was observed in wild type mice tissue sample. Double-overexpression of DC-SIGN and DC-SIGN neckless in Caco2 cells significantly decrease microbeads engulfing. Thus, EFABP associates differential transcription of DC-SIGN isoforms, then differential engulfing of antigens. According to the results of our previous studies, EFABP-Galectin4 complex might work as a M cell transcytosis enhancer. Microbeads engulfing time-lapse observation of EFABP-Galectin4-DC-SIGN triple-overexpression Caco2 also supported EFABP functional association of transcytosis. Further examinations were carried out to clarify precise mechanisms of EFABP induced M cell transcytosis.

(COI: No)

P1-178

RhoC GTPase regulates phagosome formation through mDia1 promoting actin assembly during FcγR-mediated phagocytosis in macrophges

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Phagosome formation is a complicated process that requires precisely regulated actin reorganization. Here, we demonstrate that RhoC GTPase is a crucial regulator of Fc γ R-mediated phagocytosis in macrophages. Our live-cell imaging analysis revealed that RhoC is specifically recruited to the phagocytic cups along the surface of IgG-opsonized erythrocytes (IgG-Es). RhoC silencing by RNA interference (RNAi) or the expression of GDP- or GTP-bound mutant of RhoC inhibited the rate of phagocytosis of IgG-Es. During the phagocytosis, actin-driven pseudopod extension to form phagocytic cups was severely impaired in cells expressing GTP-bound mutant RhoC-G14V, which increases cortical F-actin. mDia1, a Rho-dependent actin nucleation factor, and RhoC were colocalized at the phagocytic cup. In addition, coexpression of mDia1 along with GTP-bound RhoC-G14V or expression of constitutively active mDia1 had a drastic inhibitory effect on the uptake of IgG-Es. These data suggest that RhoC regulates phagosome formation by actin cytoskeletal remodeling via mDia1.

(COI: No)

P1-179

Rab10-positive macropinosome-like structures provide a novel endocytic pathway

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Macropinocytosis is the most effective way for cells to ingest large amounts of extracellular fluid. Its processes consist of membrane ruffling, circular ruffle (macropinocytic cup) formation and then separation form the plasma membrane as macropinosomes by cup closure. Generally macropinosomes fuse with lysosomes to degrade its contents. Recently we found that Rab10 is localized in some of macropinosome-like structures including circular ruffles in RAW264 macrophage cells. In this study, we characterized Rab10-positive macropinosome-like structures. It was observed by live cell imaging using a confocal microscope that most of Rab10-positive macropinosome-like structures disappeared in 1-5 minutes after the onset. It was frequently found that tubular structures extended from Rab10-positive macropinosome-like structures towards the perinuclear region. It was previously reported that PI(3, 4, 5)P_3 accumulates in the membrane of the formation of cup, and PI3K inhibitor, LY294002 prevents cup closure. However, formation Rab10-positive macropinosome-like structures and tubulation were not prevented by LY294002. Moreover, PI(3, 4, 5)P_3 probe, Akt-PH was not recruited to Rab10-positive macropinosome-like structures. These results suggested that Rab10-positive macropinosome-like structures may be a novel endocytic pathway that is distinct from canonical macropinocytosis.

(COI: No)

P1-180

Proteome analysis of brush border membrane fraction of small intestinal of ezrin knock-down mouse

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Ezrin is an actin binding protein which cross-links membrane proteins and actin cytoskeleton directly or indirectly through PDZ domain-containing scaffold protein. It is mainly expressed at the brush border membrane (BBM) of gastrointestinal tract, and is involved in the construction of microvilli structure and functional expression of membrane transporters at the cell surface. It was reported that the loss of ezrin disrupted the formation of apical membrane complexes. To precisely study the roles of ezrin on the expression of membrane proteins at the BBM, here we prepared the BBM fractions of small intestines from wild-type and ezrin knockdown ($Vil2^{kd/kd}$) mice, and analyzed them by LC-MS/MS and compared their proteomic patterns. In the jejunum, and ileum of $Vil2^{kd/kd}$ mice, the villus structure was maintained and the proteomic analysis showed that the expression of NHERF1 was down-regulated at the BBM. NHERF3 (PDZK1) was also down-regulated at the BBM of their jejunum. In addition, the expression of PEPT1 (Slc15a1) and SMCT1 (Sodium monocarboxylate transporter 1) (Slc5a8), which contain a PDZ domain-binding motif, was down-regulated at the BBM. Multidrug resistance protein 1 (MDR1) was up-regulated at the BBM of their jejunum and ileum in the $Vil2^{kd/kd}$ mice. On the other hand, CFTR and NHE3 were not detected in the BBM fraction by mass spectrometry although these proteins were assumed to be assembled with ezrin and located at the BBM.

(COI: No)

P1-181

The expression and localization of VAMP5 protein in the kidney

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Vesicle-associated membrane protein 5 (VAMP5) is a member of the SNARE protein family, which is generally thought to regulate the docking and fusion of vesicles with their target membranes. It has been reported that the mRNA of VAMP5 is preferentially expressed in cultured skeletal muscle cells. But the detailed expression and function of VAMP5 protein was unclear. Our study showed the expression of VAMP5 was detected in various organs by western blotting and immunohistochemistry. In this study, we found that VAMP5 was also expressed in kidney. The localization of VAMP5 in kidney was vasa recta, where the columns of capillaries in the medulla. We are now investigating the function of VAMP5 in kidney.

(COI: No)

P1-182

Function of a t-SNARE protein SNAP23 in exocrine and endocrine pancreas

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Fusion between secretory granules and plasma membranes is crucial for the exocytosis of hormones and enzymes in diverse tissues. Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) are essential for the secretory granule fusion. Synaptosomal-associated protein of 23 kDa (SNAP23), a ubiquitously expressed homologue of SNAP25, is known to be a target-SNARE protein, and forms a ternary complex with the other SNARE proteins VAMP and Syntaxin to fuse the membranes. Though the function of SNAP25 in the release of neurotransmitter has been clearly defined *in vivo*, the function of SNAP23 *in vivo* is largely unknown. To determine the function of SNAP23, we generated SNAP23 knockout (KO) mice. SNAP23 homozygous mutant mice (-/- and geo/geo) were embryonic lethal before 8.5 dpc. Thus, SNAP23 is essential for embryonic development. To know the function of SNAP23 in secretory cells, we generated pancreatic exocrine- or endocrine-specific knockout mice by Cre-loxP system. The exocrine-specific KO mice showed decreased fusion of zymogen granules, but the endocrine-specific KO mice showed increased fusion of insulin granules. These results suggested that SNAP23 has opposite roles in the secretion mechanisms of exocrine and endocrine pancreas.

(COI: No)

P1-183

ADAMTS9 /GON-1 regulates insulin secretion and insulin signaling

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ADAMTS9 is a metalloprotease that cleaves components of the extracellular matrix and is also implicated in transport from the ER to the Golgi. It has been reported that an ADAMTS9 gene variant is associated with type 2 diabetes. However, the molecular mechanisms of ADAMTS9 on the beta cell and peripheral tissues are unknown. First, we investigated how GON-1, the *C. elegans* homolog of ADAMTS9, is involved in the type 2 diabetes by using *C.elegans*. INS-7 and DAF-28 encode insulin-like proteins that are secreted from neurons in the wild type background. INS-7 and DAF-28 were accumulated in neurons in *gon-1(tm3146)* mutant background. To investigate the role of GON-1 in peripheral tissues, we examined the subcellular localization of DAF-16, the *C. elegans* homolog of FOXO. DAF-16/FOXO was present in both the nucleus and the cytoplasm in wild-type animals. DAF-16/FOXO was exclusively localized to the nucleus in peripheral tissues in *gon-1* mutant background. Next, we investigated how ADAMTS9 is involved in the type 2 diabetes by using mammalian cell lines. Glucose-stimulated insulin secretion was gradually inhibited by depletion of ADAMTS9 in the INS-1 cells, a glucose-sensitive pancreatic beta-cell line. Depletion of ADAMTS9 decreased insulin-stimulated glucose uptake in differentiated 3T3-L1-derived adipocytes and differentiated C2C12-derived skeletal muscle cells. Translocation of GLUT4 to the plasma membrane was impaired by depletion of ADAMTS9 in differentiated 3T3-L1. Our data suggest that ADAMTS9/GON-1 is involved in insulin secretion from insulin secretory cells and insulin signaling at the peripheral tissues.

(COI: No)

P1-184

Regulation of CFTR Cl channels by adenosine in pancreatic duct cells

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Introduction: Pancreatic acini secrete ATP and nucleotide-modifying enzymes that include CD39 and CD73. Adenosine, the end product of ATP, stimulated transepithelial ion transport through cystic fibrosis transmembrane conductance regulator (CFTR) Cl channels in pancreatic duct cell monolayer. However, mechanism of the regulation has not been extensively investigated.
Objectives: The present study aimed to clarify the regulation of Cl channels by adenosine in pancreatic duct cells.
Methods: We measured whole-cell current in human pancreatic duct cells (Capan-1) using gramicidin-perforated patch techniques.
Results: The application of adenosine induced a sustained inward current at 83 mV with K_d value of 10 μM. BAY 60-6583, an adenosine A_{2B} receptor agonist, increased the inward current, which was inhibited by a CFTR Cl channel inhibitor (CFTRinh-172). The current response to BAY 60-6583 was observed in 68% of the cells tested. When chloride was substituted with equimolar glutamate in the bathing solution, the reversal potential of the current-voltage curve significantly shifted from 31 to 5 mV, indicating that membrane conductance was chloride selective. The intracellular chloride activity, calculated with the Nernst equation using extracellular chloride activity and the reversal potential, was 51 mM. The inward current induced by BAY 60-6583 was also observed in a bathing solution in which sodium was replaced with *N*-methyl-D-glucamine.
Conclusion: These results indicated that the adenosine A_{2B} receptor mediated the increase in anion transport through CFTR Cl channels in Capan-1 cells.

(COI: No)

P1-185

Bicarbonate transport in interlobular pancreatic ducts isolated from cystic fibrosis mice

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Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations in CFTR gene. Pancreatic dysfunction in CF is characterized by low volume and less alkaline pancreatic juice. In this study, we analyzed HCO_3^- transport in interlobular pancreatic ducts isolated from deltaF mouse, a cystic fibrosis mouse model in which F508del mutation was introduced in the mouse Cftr. Interlobular pancreatic duct segments (diameter: ~100 μm) were isolated by microdissection. The bath and lumen were perfused separately with HCO_3^--buffered solutions. Intracellular pH (pH_i) was measured at 37°C in duct cells loaded with BCECF. In isolated pancreatic ducts from DF/DF mice, basal level of pHi was significantly ($p<0.05$) higher (by ~0.06 pH unit) compared with ducts from wild-type (wt/wt) mice. Stimulation with forskolin (1 μM) caused significant ($p<0.05$) elevation of pHi by 0.049 ± 0.008 unit (mean ± SE, n=6) in DF/DF ducts but not in wt/wt ducts. Under stimulation with forskolin, $NH4^+$ pulse (20 mM, 2 min) followed by Na^+ removal from bath and lumen caused intracellular acid-loading to pH 6.8~6.9. Under high-K^+ (70 mM) in the bath, restoration of Na^+ to the luminal solution caused significantly ($p<0.05$) faster increase of pH_i in DF/DF ducts compared to wt/wt ducts. In summary, higher level of pHi in DF/DF ducts is consistent with impaired HCO_3^- secretion across the apical membrane. The present data also suggest that the activity of Na^+-dependent HCO_3^- absorption across the apical membrane is enhanced in DF/DF ducts.

(COI: No)

P1-186

Antioxidant signaling involving the microtubule motor KIF12 is an intracellular target of nutrition excess in beta cells

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Beta cell injury due to oxidative stress is a typical etiology of diabetes caused by nutritional excess, but its precise mechanism remains largely elusive. Here, we demonstrate that the microtubule motor KIF12 mediates an antioxidant cascade in beta cells as an intracellular target of excess fat intake or "lipotoxicity." KIF12 knockout mice suffer from hypoinsulinemic glucose intolerance due to increased beta cell oxidative stress. Using this model, we identified an antioxidant signaling cascade involving KIF12 as a scaffold for the transcription factor Sp1. The stabilization of nascent Sp1 appeared to be essential for proper peroxisomal function by enhancing Hsc70 expression, and the pharmacological induction of Hsc70 expression with teprenone counteracted the oxidative stress. Because KIF12 is transcriptionally downregulated by chronic exposure to fatty acids, this antioxidant cascade involving KIF12 and Hsc70 is proposed to be a critical target of nutritional excess in beta cells in diabetes.

(COI: Properly Declared)

P1-187

Modeling Analysis of Glucagon-like peptide-1 (GLP-1)-induced Inositol 1, 4, 5-trisphosphate Receptor (IP3R)-Mediated Ca^{2+} liberation in Pancreatic β -Cells

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Upon elevation of plasma glucose concentration, pancreatic β-cells generate bursts of action potentials and produce cyclic changes in intracellular calcium concentration ($[Ca^{2+}]$) regulating pulsatile insulin release. GLP-1 increases cAMP levels and synergistically enhances glucose-dependent insulin secretion. Further rise of $[Ca^{2+}]$, in forms of Ca^{2+} transients and oscillations, achieved by mobilization of intracellular stores through IP3R was suggested to be part of the fundamental mechanisms by which cAMP effectors amplify insulin release in murine β-cells. The molecular mechanisms as well as the intracellular conditions to evoke GLP-1-induced Ca^{2+} liberations, however, have not still been well elucidated. Here we developed a mathematical model of IP3R and reconstructed GLP-1-induced Ca^{2+} transients and oscillations in a simplified cellular model. Simulation studies and mathematical analyses were then applied to investigate the mechanisms of the IP3R-mediated Ca^{2+} mobilizations in pancreatic β-cells. Results indicated that Ca^{2+} transients and oscillations were produced by positive feedback involving Ca^{2+}-dependent activation of the channel. A slower rate of Ca^{2+}-dependent inactivation was revealed to provide a remarkable contribution to determine the time course of the decay in Ca^{2+} transients. Interestingly, Ca^{2+}-dependent inactivation of the channel was the key to driving and pacing Ca^{2+} oscillations whereas fast rate of Ca^{2+}-dependent activation amplifies the signal.

(COI: No)

P1-188

Analysis of changes in the Ca^{2+} concentration in the endoplasmic reticulum during Ca^{2+} oscillations in mammalian eggs

Kikuchi, Takashi; Murata, Takasuke; Shirakawa, Hideki (*Dept Eng Sci, Univ Electro-Comm, Tokyo, Japan*)

Repetitive increases in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$), or Ca^{2+} oscillations, are induced in mammalian eggs by the fusing spermatozoa, and trigger a series of events leading to egg activation. Each Ca^{2+} transient in the oscillations is due to Ca^{2+} release from the endoplasmic reticulum (ER) through inositol 1, 4, 5-trisphosphate receptor/Ca^{2+} channels. To understand the mechanism of Ca^{2+} oscillations, therefore, the information about the relation between the Ca^{2+} concentration in the ER ($[Ca^{2+}]_{ER}$) and $[Ca^{2+}]_{cyt}$ is essential. In the present study, we measured the changes in $[Ca^{2+}]_{ER}$ during Ca^{2+} oscillations in mouse eggs induced by the sperm-borne egg-activating protein, phospholipase C ζ, using a genetically coded Ca^{2+} probe, D1ER. By simultaneous monitoring of $[Ca^{2+}]_{ER}$ and $[Ca^{2+}]_{cyt}$, it was revealed that the typical time course of the change in $[Ca^{2+}]_{ER}$ at each Ca^{2+} transient consists of four consecutive phases: a fast decrease, a flat bottom, a fast increase, and a much slower increase, each of which corresponds to a fast increase, a slow decrease, and a fast decrease, and a much slower increase in $[Ca^{2+}]_{cyt}$, respectively. The rates of the fast increase and decrease in $[Ca^{2+}]_{ER}$ were both affected by the ER Ca^{2+} pump inhibitor, thapsigargin. The rate of the slow increase was not inhibited by thapsigargin, but was dependent on the extracellular Ca^{2+} concentration. Results of the experiments to elucidate the effect of Ca^{2+}-buffering proteins in the ER on the Ca^{2+} dynamics will also be discussed.

(COI: No)

P1-189

Down-regulation of Ca^{2+}-activated Cl^- channel TMEM16A by the inhibition of histone deacetylase in human breast cancer cell line YMB-1

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The Ca^{2+}-activated Cl^- channel TMEM16A plays an important role in facilitating cell growth and metastasis of TMEM16A-expressing cancer cells. Histone deacetylase HDAC inhibitors (HDACis) are useful agents for cancer therapy, but, it remains unclear whether ion channels are epigenetically regulated by them. Utilizing real-time PCR, Western blot and whole-cell patch clamp assays, we found a significant decrease in TMEM16A expression and its functional activity induced by vorinostat, a pan-HDACi in TMEM16A-expressing human breast cancer cell line YMB-1. Pharmacological blockade of HDAC3 by 1 μM T247, a HDAC3-selective HDACi elicited a large decrease in TMEM16A expression and functional activity in YMB-1, and pharmacological blockade of HDAC2 by AATB (300 nM) elicited partial inhibition of TMEM16A expression (about 40 %). In addition, siRNA-induced inhibition of HDAC3 elicited a large decrease in TMEM16A transcript in YMB-1. Taken together, in malignancies with a frequent gene amplification of TMEM16A, HDAC3 inhibition is suggested to exert suppressive effects on cancer cell viability via a downregulation of TMEM16A.

(COI: No)

P1-190

A ventricular cell model refined on Ca^{2+}-induced Ca^{2+}-release

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The graded Ca2+ release via ryanodine receptors (RyRs) is dependent on the extent of activation of the L-type Ca2+ channel current (ICaL) within the dyadic junction. However, this local control of Ca2+ release is still not achieved in most of cardiac cell models developed on the desk-top computer level. Mostly, the Ca2+-induced Ca2+-release occurred in an all-or-none manner because a single common pool was assumed for both ICaL and RyR Ca2+ fluxes. We adopted the model of Ca2+ induce Ca2+ release (CICR) based on local control theory (Hinch et al. 2004) in the guinea pig ventricular cell model. This CICR model was improved by incorporating the experimental large Ca2+ gradient recorded near the Ca2+ releasing site, and by removing the RyR inactivation. The new cell model demonstrated that CICR was terminated through the local Ca2+ depletion in the sarcoplasmic reticulum. Furthermore, the graded Ca2+ release during voltage clamp pulse was observed in the presence of local Ca2+ accumulation in junction space. The detailed mechanism of induction decay of CICR and comparison of the new ventricular cell model with previous animal ventricular cell models will be presented.

(COI: No)

P1-191

An activator of TRPM7, naltriben, accelerates Mg^{2+} influx in rat ventricular myocytes

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To estimate the Mg^{2+} influx rate, we measured cytoplasmic free Mg^{2+} concentration ($[Mg^{2+}]_i$) in rat ventricular myocytes with a fluorescent indicator furaptra (mag-fura-2). $[Mg^{2+}]_i$ was first lowered by depleting the cells of Mg^{2+}, and was subsequently recovered to the basal level in Ca^{2+}-free Tyrode's solution containing 1 mM Mg^{2+}. The time course of the $[Mg^{2+}]_i$ recovery was generally well described by a single exponential function, and was analyzed as the Mg^{2+} influx rate. The rate of Mg^{2+} influx was, on average, 0.27 ± 0.04 μM/s with the initial $[Mg^{2+}]_i$ at 0.35 ± 0.02 mM (n=10). We studied the effect of naltriben, a δ opioid receptor antagonist, recently identified as an activator of the TRPM7 channel [1]. Application of naltriben (50 μM) significantly increased the rate of Mg^{2+} influx, 0.57 ± 0.12 μM/s, with similar initial $[Mg^{2+}]_i$ at 0.37 ± 0.02 mM (n=7). In the presence of 50 μM naltriben, the $[Mg^{2+}]_i$ recovery often had a transient overshoot; $[Mg^{2+}]_i$ reached the level higher than the basal level before it slowly decreased to the basal level. In combination with our previous results that inhibitors of the TRPM7 channel slowed the rate of Mg^{2+} influx [2], the present results suggest a major role of the TRPM7 channel as a physiological Mg^{2+} influx pathway in cardiac myocytes.

[1] Hofmann T, et al. Pflugers Arch. DOI: 10.1007/s00424-014-1488-0, 2014
[2] Tashiro M, Inoue H, Tai S, Konishi M. J Physiol Sci 64:S225, 2014

(COI: No)

P1-192

Role of an intestinal ion transport in salt-sensitive hypertension

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The specific mechanisms by which high-salt diet lead to the elevation of blood pressure have been elucidating. High oral salt intake are firstly sensed by the brain, to activate renin-angiotensin-aldosterone system and enhance the production of endogenous ouabain. The secretion of cardiotonic steroid (CTS) from adrenals is chronically enhanced, and then effects on target peripheral organs by both inhibiting the pump activity of Na^+/K^+-ATPase (NKA) and activating the intracellular signaling pathway via NKA. In the proximal renal tubules (PRT), it enhances the trafficking of basolateral NKA and luminal Na^+/H^+ exchanger (NHE) 3 to stimulate natriuretic response, which is suppressed in Dahl salt-sensitive (DSS) hypertensive rats. There is many similarities in the ion transport mechanism between the intestine and the PRT including the trafficking of NHE3. As the first step to elucidate whether the intestine is one of the targets of CTS, in the present study, we compared the effect of high-salt diet on intestinal NKA activity between DSS hypertensive rats and salt-insensitive Sprague-Dawley (SD) rats using Mucosa-submucosal preparations mounted on the Ussing chamber. Short-circuit current and tissue conductance were measured as indices of transepithelial ion transport and permeability. High-salt diet increased ion transport stimulated by ouabain less in DSS hypertensive rats than in SD rats. The more selective study to measure basolateral NKA activity will be performed by treating ionophore to permeabilize in the absence of mucosal Na^+.

(COI: No)

P1-193

Volume-sensitive anion channels in melanoma cells before and after tumor formation

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Melanoma is one of the most aggressive malignancies commonly associated with poor prognosis for patients; it is characterized with a high level of drug resistance and successful escape from apoptosis. Volume-sensitive outwardly rectifying anion channel (VSOR) is known to play a key role in cell proliferation and in apoptotic cell death. However, it is poorly characterized in melanomas. We studied the phenotype of VSOR in two melanoma cell lines: parental B16 and KML (patent UZ IAP 02729), which was obtained by continuous culture of excised primary lung tumors from mice intravenously injected with B16. When inflated by using slightly hypertonic pipette solutions, both cell types responded with swelling accompanied with robust activation of anionic currents exhibiting the VSOR phenotype. The macroscopic current density, degree of outward rectification and sensitivity to DCPIB were indistinguishable in both cell types. However, VSOR in KML cells became activated about twice as faster, had higher selectivity to glutamate over chloride and a significantly smaller single-channel amplitude. We hypothesize that modulation of the biophysical properties of VSOR of melanoma cells by their history of tumor formation in vivo might be due to differential expression of auxiliary components of the whole channel complex.

(COI: No)

P1-194

Volume-sensitive anion channel regulates butyrate-induced apoptosis

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Butyrate is present in colonic epithelium at millimolar concentrations and involved in keeping colonic homeostasis. Although it has been demonstrated that excess of butyrate triggers cell death in colonic epithelial cells, the mechanism is poorly understood. Recently, volume-sensitive anion channel is reported to be involved in a variety of cell death. In the present study, therefore, we investigated whether the volume-sensitive anion channel contributes to the butyrate-induced cell death in mouse colonic epithelial MCE301 cells. Whole-cell patch-clamp recordings demonstrated that volume-sensitive currents after cell swelling exhibit outward rectification, time-dependent inactivation on more depolarized potentials, and anion selectivity ($I^->Br^->Cl^->F^-$). The volume-sensitive anion currents were sensitive to Cl^- channel blockers, DCPIB (2.5 μM) and NPPB (10 μM). Flow cytometry using annexin V-FITC and propidium iodide indicated MCE301 cells treated with butyrate (8 mM) for two days were in late apoptosis. Interestingly, butyrate-induced late apoptosis was inhibited by Cl^- channel blockers. In the cells, apoptotic volume decrease and caspase 3/7 activation were observed 16 h after the butyrate application, and these effects were also suppressed by Cl^- channel blockers. Our results suggest that the volume-sensitive anion channel is essential in the butyrate-induced apoptosis in mouse colonic epithelial MCE301 cells.

(COI: No)

P1-195

Hypotonicity-activated cation currents in the principal cells of isolated rat Kidney cortical collecting Ducts

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We have previously demonstrated that the hypotonicity-induced Ca^{2+} entry was inhibited by a voltage-gated Ca^{2+} channel inhibitor, Nicardipine in the principal cells of rat cortical collecting ducts (CCDs). However, electrophysiological properties and molecular identity of the hypotonicity-induced Ca^{2+} entry pathway are still unknown. In this study, we performed whole-cell voltage clamp recording to confirm whether a cation conductance is activated in response to the hypotonicity in the principal cells of rat CCDs. To minimize K^+ and Cl^- currents, whole-cell recordings were carried out using NMDG-methansulfonate pipette and Na-gluconate bath solution. When exposed to hypotonic solution, whole-cell current amplitude at -80 mV was gradually increased. The activation of whole-cell currents was also observed using the Na^+ free bath solution containing 5 mM Ca^{2+}. The hypotonicity-activated wholec-cell current was inhibited by the application of Nicardipine but not influenced by either Gd^{3+} or amiloride. Although RT-PCR analysis showed the presence of transcripts of T-type Ca^{2+} channel $\alpha 1_G$ subunit, but not L-type Ca^{2+} channel $\alpha 1_C$ subunit in rat CCD, a T-type Ca^{2+} channel blocker, Ni^{2+} did not change the hypotonicity-induced current activation. These data suggest that a Nicardipine sensitive cation conductance, which is activated by hyotonicity is present in the principal cells of rat CCDs. We will further characterize the current to elucidate the molecular identity of this current.

(COI: No)

P1-196

Effects of extracellular phosphates on voltage-gated H+ channels in RAW-derived osteoclast-like cells

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Osteoclasts are highly differentiated bone-resorbing cells and play a significant role in bone remodeling. In the resorption pit, formed between the plasma membrane of osteoclasts (the ruffled membrane) and the bone surface, the concentrations of Ca^{2+} and inorganic phosphates (Pi) are increased, according to degradation of hydroxyapatite by a large amount of protons secreted from osteoclasts. The rise in the extracellular Ca^{2+} level inhibits osteoclastic bone resorption, but the effects of extracellular Pi on osteoclast functions, particularly on H^+ fluxes in the membrane, are largely unknown. We investigated the effects of extracellular Pi on the voltage-gated H^+ channels in osteoclast-like cell generated from a macrophage cell line (RAW264) using the whole-cell recordings. In the presence of extracellular Na^+, Pi (1-20 mM) increased the H^+ currents reversibly under the condition where the Na^+/H^+ exchanger was inhibit by its blocker, amiloride. The enhancement was observed even in the absence of intracellular ATP. The reversal potential of the H^+ channels shifted slightly to more positive voltages by Pi, suggesting that the potentiation was not due to increases in the driving force for protons. In the absence of Na^+, 20 mM Pi increased the H^+ currents at pH 5.5-6.5. These data suggest that extracellular Pi might modify the H^+ channel properties in both presence and absence of extracellular Na^+, leading to increases in the channel activities in osteoclasts.

(COI: No)

P1-197

An acid-inducible proton influx mechanism in the plasma membrane of osteoclasts

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Osteoclasts dissolve bone tissue by secreting acids and proteolytic enzymes from the plasma membrane facing bone tissue (the ruffled membrane) into the resorption pit. Consequently, the plasma membrane faces to highly acidic extracellular environments (~pH 4), and the pH gradient across the plasma membrane could generate a large driving force for protons entering into the cells. However, the proton influx mechanism in osteoclasts exposed to strong acids is largely unknown. In murine osteoclasts derived from RAW264, we identified inward currents activated by decreasing the extracellular pH lower than 5.5. The currents were characterized by a high proton-selectivity, a slight inward rectification and insensitivities to amiloride and ruthenium red, blockers for acid-sensitive cation channels, ASIC and TRP channels, and, to DIDS, a blocker for Cl^- channels. The acid-inducible proton influx decreased the intracellular pH near the plasma membrane, which was monitored by the reversal potentials of voltage-gated proton channels coexisted in the same membrane, even when V-ATPases and Na^+-H^+ exchangers were functional. These results suggested that osteoclasts may possess a proton-selective pathway which could mediate proton influx upon severe extracellular acidification. The acid-inducible proton influx may regulate the pH of the resorption bit by balancing the rates of proton pumping out by V-ATPases or may modify osteoclast functions through intracellular acidification.

(COI: No)

P1-198

Regulation of vessel formation by synthetic peptide derived from activator of G-protein signaling 8

Mamun, Abdullah; Hayashi, Hisaki; Suzuki, Hiroko; Sakima, Miho; Sato, Maki; Nishimura, Naoki; Inukai, Yoko; Iwase, Satoshi; Sato, Motohiko (*Dept Physiol, Aichi Med Univ, Nagakute, Japan*)

Heterotrimeric G-proteins are essential signal transducers involved in many human diseases. Previously, we identified an ischemia-inducible G-protein activator, activator of G-protein signaling 8 (AGS8) from angina model of the rat heart, which bound heterotrimeric G-protein $\beta\gamma$ subunit ($G\beta\gamma$). AGS8 is involved in hypoxia-induced apoptosis of cardiomyocytes and vessel formation of endothelial cells. Previously, we have developed a synthetic peptide (AGS8 peptide) for AGS8-specific signal intervention, based amino acid sequences of biding domain of AGS8 to $\beta\gamma$. AGS8 peptide successfully protected cultured cardiomyocytes from hypoxia-induced apoptosis. Here, we examined the effects of AGS8 peptide and Gallein, a small compound designed as universal $G\beta\gamma$ signal inhibitor, on tube formation of HUVEC. AGS8 peptide inhibited vascular endothelial growth factor (VEGF) induced tube formation of HUVEC in vitro (20 μg/ml: 46.4% ± 4.2, $p<0.01$ vs negative control, mean ± SEM). AGS8 peptide also inhibited VEGF-induced phosphorylation of VEGF receptor type 2 (Tyr 996; 26.7 ± 3.2 %, $p<0.05$, Tyr 1175; 19.8 ± 4.5 % vs negative control, $p<0.05$). In contrast with AGS8 peptide, Gallein did not blocked VEGF-induced tube formation and phosphorylation of VEGF receptor. These data indicate an advantage of specific intervention of AGS8-mediated signal by synthetic peptide and a potential of AGS8 as a therapeutic target for cardiovascular diseases.

(COI: No)

P1-199

Activator of G-protein signaling 8 is required for angiogenesis in vascular endothelial cells

Hayashi, Hisaki; Mamun, Abudullah; Suzuki, Hiroko; Sakima, Miho; Sato, Maki; Nishimura, Naoki; Inukai, Yoko; Iwase, Satoshi; Sato, Motohiko (*Dept Physiol, Aichi Med Univ, Nagakute, Japan*)

We have previously identified receptor-independent G-protein regulator, activator of G-protein signaling (AGS) 8, from a rat heart model of repetitive transient ischemia. AGS8 expression was up-regulated in rat ischemic heart, and directly interacted with G-protein $\beta\gamma$ subunit ($G\beta\gamma$). AGS8 played a pivotal role in the hypoxia-induced apoptosis of cardiomyocytes by regulating $G\beta\gamma$ signaling. Since collateral arteries were significantly developed in the experimental model, we hypothesized that AGS8 was involved in vascular formation. Here, we analyzed roles of AGS8 in vascular endothelial cells (ECs). AGS8 knockdown by siRNA inhibited VEGF-stimulated tube formation of HUVEC on matrigel (27.0 ± 4.8 % of control, $p<0.01$, mean ± SEM). MTT assay revealed that AGS8 knockdown also inhibited VEGF-induced cell proliferation (30.5 ± 3.7 % of control, $p<0.01$, mean ± SEM). AGS8 knockdown significantly suppressed VEGF-induced phosphorylation of VEGF receptor type 2 (VEGFR2), ERK1/2 and p38/MAPK. Interestingly, FACS analysis demonstrated that AGS8 knockdown reduced VEGFR2 localization on the cell surface. Our data first indicate that G-protein regulator is involved in VEGF-mediated angiogenesis by influencing distribution of VEGFR2 and activation of VEGFR2 signal. Therefore, AGS8 is a potential therapeutic target of pathological angiogenesis in ECs, as well as of hypoxia-induced apoptosis in cardiomyocytes.

(COI: No)

P1-200

Analysis of temporal-information coding mechanism of MAPK signaling

Tomida, Taichiro; Saito, Haruo (*Div. of Mol. Cell Signal., Inst. of Med. Sci., Univ. of Tokyo*)

Signaling by the conserved MAP kinase family is a major mechanism through which eukaryote cells respond properly to various extracellular stimuli and induce adaptive responses, such as gene expression, control of cell cycle, growth, and differentiation. Mechanisms and regulation of MAPK signaling have been elucidated in detail, and it is becoming possible to predict kinase activation in sillico, however, how cells induce appropriate cellular function according to the context of their surrounding environment (or stimulation type) remains unclear. Moreover, because living cells under physiological conditions are normally exposed to fluctuating environment, how cells interpret and process those temporally changing information and activate pertinent adaptive response also remains elusive. For this, we took advantage of real-time imaging of kinase activity within living cells/ cells of living animals to analyze its regulatory mechanism in situ. We performed control systems type of analysis that consist of application of a set of defined multiple stimuli to cells (input) followed by measurement of kinase response (output), from which systems properties are deduced. From this, we found that MAPK signaling exhibit a complex, non-linear response to the duration of stimulation to cells. Our results suggested that MAPK signaling deciphers the different temporal pattern of input stimulation. Furthermore, a combination of imaging analysis with mathematical modeling revealed that the complex input-output relation of the kinase signaling can be explained by a fairly simple regulatory mechanism.

(COI: No)

P1-201

This poster presentation was withdrawn.

P1-202

Mechanosensitive ATP release from hemichannels causes acceleration of wound healing in keratinocytes via Ca^{2+} influx through TRPC6 channels

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The cutaneous wound healing (gap closing) was accelerated by a mechanical stimulation, stretch, and impaired in TRPC6-KO mice. Here we elucidated how the mechanical force and TRPC6 channels contribute to the wound healing. HaCaT keratinocytes were cultured on an elastic chamber and treated 1 day with hyperforin, known as a traditional herbal medicine and also a TRPC6 activator. At 3 h after making scratching, ATP release and intracellular Ca^{2+} response by stretch were determined by live-imaging using luciferin-luciferase luminescence and fluo-8 fluorescence, respectively. ATP release was observed only from foremost cells of leading edge of wounded area and it caused Ca^{2+} waves spreading to the behind cells. The Ca^{2+} response and the acceleration of wound healing were inhibited by a diphosphohydrolase, apyrase, a P2Y antagonist, suramin, a hemichannel blocker, CBX and a PIP_2 analog, diC8-PIP_2. In addition, hemichannel permeable dye calcein entered to only ATP-releasing cells. These results suggested that stretch-accelerated wound closure was due to ATP release via hemichannels from the foremost cells and subsequent Ca^{2+} waves in the behind cells mediated by TRPC6 activation.

(COI: No)

P1-203

Dipalmitoleoyl-phosphatidylethanolamine attenuates insulin signaling by enhancing PP2A and PTP1B activities

Tsuchiya, Ayako; Kanno, Takeshi; Nishizaki, Tomoyuki (*Div Bioinform, Dept Physiol, Hyogo College Med, Hyogo, Japan*)

The phospholipid phosphatidylethanolamine is implicated in the regulation of a variety of cellular processes. The present study investigated the effect of phosphatidylethanolamines such as 1, 2-diarachidonoyl-*sn*-glycero-3-phosphoethanolamine (DAPE), 1, 2-dilinoleoyl-*sn*-glycero-3-phosphoethanolamine (DLPE), 1, 2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), and 1, 2-dipalmitoleoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) on protein phosphatases, Akt1/2 activity, GLUT4 mobilizations, and glucose uptake into cells. Of the investigated phosphatidylethanolamines, DLPE and DPPE significantly enhanced activities of protein phosphatase 2A (PP2A) and protein tyrosine phosphatase 1B (PTP1B). DPPE inhibited insulin-induced phosphorylation of insulin receptor, insulin receptor substrate 1 (IRS-1), Akt1/2, ERK1/2, and mammalian target of rapamycinm (mTOR) in differentiated 3T3-L1-GLUT4myc adipocytes. DPPE also inhibited insulin-stimulated GLUT4 translocation to the cell surface and reduced insulin-stimulated glucose uptake into adipocytes. Taken together, the results of the present study indicate that DPPE serves as an enhancer of PP2A and PTP1B, causing reduction of Akt1/2 activity as a result from inhibiting insulin receptor and IRS-1 or mTOR, and then leading to suppression of GLUT4 translocation to the cell surface and glucose uptake into adipocytes.

(COI: No)

P1-204

The voltage-dependence and mechanism of RGS4-mediated regulation on the M2 muscarinic receptor-activated K^+ currents

Chen, Ishan; Furutani, Kazuharu; Inanobe, Atsushi; Kurachi, Yoshihisa (*Dept Pharmacol, Grad Sch Med, Osaka Univ, Osaka, Japan*)

The regulator of G-protein signalling (RGS) proteins are a family of well-known GTPase-activating proteins that negatively regulate G-protein cycle. We have found that cardiac predominant subtype, RGS4, plays an important role in modulating the M2 muscarinic receptor (M2R)-activated G-protein-gated inwardly rectifying K^+ (K_G) currents. However, the mechanism of RGS4-mediated regulation still remains unclear. Here we show that RGS4 is essential for the voltage-dependent response of K_G currents upon M2R agonists. In rat atrial myocytes, M2R partial agonist pilocarpine-evoked K_G currents showed a decrease in current amplitude during membrane hyperpolarization. In a *Xenopus* oocyte expression system, we observed a similar voltage-dependent response of pilocarpine-evoked current in the presence of RGS4, while it lacked such voltage-dependent property in the absence of RGS4. We found that RGS4 suppressed the pilocarpine-evoked K_G currents in a pilocarpine concentration-dependent manner. Such RGS4-mediated regulation was enhanced at hyperpolarized potentials. We also found that the relative efficacy of pilocarpine to ACh changed upon membrane voltages. Charged residues of M2R modulated the voltage-dependence of RGS4-mediated regulation on K_G currents. These findings help us to understand the molecular components and mechanism underlying the RGS4-mediated regulation on the M2R-activated physiological responses.

(COI: No)

P1-205

Cell line dependency of cesium ion induced suppression of cellular proliferation

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Since aftermath of the 2011 Tohoku earthquake and tsunami, cesium (Cs) as a radioisotope became popular, but on the other hand, intracellular reaction of Cs^+ ion is still little known. It is already known that Cs is similar to potassium (K). And it is also well known that K^+ channel plays an important role in tumor cell proliferation. We previously demonstrated that Cs^+ inhibited HeLa cells proliferation. In this study, we established a new primary culture system of rat airway fibroblast cell (RAWF). RAWF was obtained from rat trachea by enzymatic digestion using protease type XIV in 4℃ for over night. EC50 of Cs^+ inhibiting cell proliferation was 4 ± 1 mM (mean ± SD) on HeLa cells, on the other hand, 19 ± 9.5 mM on RAWF. This imply as a possibility that Cs^+ has a stronger cell proliferation inhibiting effect on carcinoma cells than on normal cells. In addition, we will report the inhibiting effect of Cs^+ on the cell proliferation of some other cell lines such as B16, SH-SY5Y and so on.

(COI: No)

P1-206 (AP-1)

TRPM2 protects mice against polymicrobial sepsis by enhancing bacterial clearance

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TRPM2 is an oxidative stress-activated nonselective Ca^{2+} permeable channel abundantly expressed in macrophages to regulate production of inflammatory mediators. However, the role and mechanism of TRPM2 in polymicrobial sepsis remains unclear. Using CLP-induced polymicrobial sepsis model, Trpm2-KO mice had increased mortality compared with wild-type (WT) mice. The increased mortality was associated with increased bacterial burden, organ injury, and systemic inflammation. TRPM2-mediated Ca^{2+} influx plays an important role in LPS or CLP-induced HO-1 expression in macrophage. HO-1 up-regulation decreased bacterial burden both in WT BMDMs and in CLP-induced septic WT mice. Disruption of TRPM2 decreased HO-1 expression and increased bacterial burden in BMDMs. Interestingly, pretreatment of Trpm2-KO BMDMs with HO-1 inducer markedly increased HO-1 expression and decreased bacterial burden. Moreover, pretreatment of Trpm2-KO mice with HO-1 inducer reversed the susceptibility of Trpm2-KO mice to sepsis by enhancing bacterial clearance. In addition, septic patients with lower monocytic TRPM2 and HO-1 mRNA levels had a worse outcome compared with septic patients with normal monocytic TRPM2 and HO-1 mRNA levels. TRPM2 levels correlated with HO-1 levels in septic patients. Our data demonstrate a protective role of TRPM2 in controlling bacterial clearance during polymicrobial sepsis possibly by regulating HO-1 expression.

(COI: No)

P1-207

Morphological studies on cell membrane permeability of amphiphilic gold nanoparticles in cultured Schwann cells and dorsal root ganglion cells

Ninomiya, Takafumi[1]; Kikuchi, Shin[1]; Niikura, Kenichi[2]; Tatsumi, Haruyuki[1] (*[1]Sapporo Med. Univ. Sch. Med., Sapporo, Japan; [2]Hokkaido Univ., Sapporo, Japan*)

Gold nanoparticles (AuNP) have been the focus of much attention as an attractive material for medical uses, such as a tool for photothermal therapy, biosensing devices and drug delivery carriers. Gold nanoparticles are usually taken into the cells by endocytosis. Niikura and colleagues have developed amphiphilic gold nanoparticles with cell membrane permeability. The gold nanoparticles were coated with ethyl ester-headed polyethylene glycol ligands (C2-Ester). To verify the cell membrane permeability of the C2-Ester AuNP into the cells, the morphology of the cultured Schwann cells and dorsal root ganglion (DRG) cells was observed by electron microscopy. The gold nanoparticles coated with C2-Ester were localized not only in the endosomes and multivesicular bodies but also in the cytosol of Schwann cells and DRG cells. The uptake of C2-Ester AuNP in the DRG cells was less than in the Schwann cells. The presence of the C2-Ester AuNP in the cytosol is evidence that the AuNP was taken up by the cell membrane permeability. Our finding regarding the advantages of ester ligands will be applicable in exploring how they deliver the various functional nanoparticles into cells. However, electron microscopy did not provide evidence in the present study indicating that the C2-Ester AuNP arrives in a nuclear pore and nucleus. The matter of how to develop amphiphilic AuNP that can reach into the nucleus is yet to be resolved.

(COI: No)

P1-208

Expression analysis of metallothionein genes and application to the production of heterologous proteins in *Tetrahymena thermophila*

Owada, Kyoko; Yanagisawa, Masaomi; Saiki, Mizuho; Tomaru, Manami (*Dep Chem and Material Sci, Gunma Natl Coll Tech, Maebashi, Japan*)

Metallothioneins (MTs) are a family of low molecular weight (>10 kD), cystein-rich, absence of aromatic amino acids, heavy metal-binding proteins. The expressions of MTs genes are induced by the presence of heavy metal such as zinc, copper, and cadmium. MTs play important roles in the maintenance of homeostasis and in the detoxification of heavy metals. Recently several studies suggested a disturbance of the zinc and copper metabolism related to autism spectrum disorders. The zinc deficiency and an excess of copper level are observed in children diagnosed with autism spectrum disordered. For the experiment of toxic compounds, *Tetrahymena* is excellent eukaryote model organism. Using *T. thermophila* the expression level of copper-inducible metallothionein genes in response to zinc and copper has been carried out by RT-PCR. *T. thermophila* was grown in PPYG medium at 25℃. Cells were treated with 2-500 μM $CuSO_4$, 2-870 μM $ZnSO_4$ for 24h. Total RNA was isolated using RNeasy Kit (Quagen) from the cells (5-8 × 10^5). AMV reverse transcriptase was used to synthesize cDNA. Template cDNA was amplified with MTT2 primers. The 5′ region of a copper-inducible metallothionein gene (MTT2) acts as a promoter, a region 1456 bp upstream of the start codon in MTT2 was amplified and ligated into a vector (pMTT2^p-EGFP). This recombinant vector introduces by electroporation into T. thermophila. MTT2 promoter, strongly induced by copper, might be effective to produce the higher level of MTs in various tissues.

(COI: No)

P1-209

Effects of food additives on human neuroblastoma-derived cells

Onoue, Sakura[1]; Higashi, Kazuyoshi[2]; Kawada, Akira[2]; Sasaki, Yasushi[3]; Takahashi, Osamu[2] (*[1]Dept. of Biosci., Col. of Sci. and Eng., Kanto Gakuin Univ. Yokohama, Japan; [2]Dept. Histol., Embryol. and Neuroanat., Kanagawa Dental Univ. Graduate sch. of Dentistry, Yokosuka, Japan; [3]Dept. of Appl. Matl. and Life Sci., Col. of Eng., Kanto Gakuin Univ. Yokohama, Japan*)

Introduction: Several reports have described the effect of food additives on cell activities. However, the details of such effect were unclear. Thus, we investigated the effects of food additives used alone or in combination on human neuroblastoma-derived cells, focusing particularly on morphological changes observed in these cells.
Materials and Methods: Tumor cells derived from human neuroblastoma (NB-1) were cultured in medium containing a single food additive or multiple food additives (aspartame, tartrazine, sodium benzoate, sodium nitrite). The morphology of these cells and the fine structure of cell surface were observed by scanning electron microscope. The effects of these food additives on degeneration or death of cells were examined by double staining using fluorescein diacetate and propidium iodide.
Results and Discussion: In this study, there was marked decrease in numbers of cell processes incubated with sodium nitrite alone, sodium benzoate alone, or multiple food additives. A statistically significant decrease in the cell survival rate was apparent in cells cultured with sodium nitrite alone, sodium benzoate alone, or multiple food additives, in comparison with cells of control. From these data, it was suggested that food additives may exert major influences on functional properties of neuroblastoma-derived cells.

(COI: No)

P1-210

Development of three color variants of super-brilliant luminescent proteins for multicolor, real-time bioluminescent imaging

Takai, Akira[1]; Nakano, Masahiro[2]; Nagai, Takeharu[1, 2, 3]; Okada, Yasushi[1] (*[1]QBiC, RIKEN, Osaka, Japan; [2]ISIR, Osaka Univ., Osaka, Japan; [3]PRESTO, JST, Tokyo, Japan*)

Since bioluminescence is free from auto-fluorescence, it has been used for quantitative analysis of gene expression and in vivo imaging. Furthermore, it is free from potential phototoxicity and is compatible with optogenetic tools. However application of bioluminescent imaging has been limited mainly by two drawbacks. Firstly, the light output from the bioluminescent protein was much dark. Secondly, its color variants have been limited, precluding multicolor imaging. In recent study, we addressed the first limitation by developing a super brilliant yellow luminescent protein, Nano-lantern (Saito et al., Nat. Commun. 2012). In this study, we have overcome the second barrier. We report the development of cyan and orange variants of Nano-lantern, both of which are even brighter than the original yellow Nano-lantern by 1.5-2.3 times. Fusions of these multicolor Nano-lanterns with a variety of subcellular localization tags showed correct localization, demonstrating their utility as imaging probes. In addition, expansion of the color palette of Nano-lanterns also enabled expression analysis of multiple genes at single cell level in embryonic stem cells, which are known to be very sensitive to phototoxicity. Furthermore, by combining split luciferase complementation with Ca^{2+}-sensing peptide (CaM-M13), we demonstrated simultaneous measurement of Ca^{2+} dynamics in the nucleus and mitochondria. These data indicates our multicolor Nano-lanterns will be excellent imaging tools for in vivo imaging, stem cell study and so on.

(COI: No)

P1-211

Special-purpose simulators for biological research: intracellular calcium dynamics

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Recent advances in live imaging have enabled visualization of spatiotemporal dynamics in living cells. However, mechanisms that orchestrate cellular dynamics have not been well elucidated. Computer simulations are necessary to elucidate these mechanisms because they allow us to predict dynamics of hypothetical models. The predicted dynamics are to be used for improving the models, compared with in vivo dynamics. Despite such importance, computer simulations are not widely used in biological research. A major reason for such limited usage is the difficulty in setting up simulations. Although many high-performance simulators are currently available for biological research, setting up simulations requires some programming-like skills in these simulators because they are designed as a general-purpose simulator. To accelerate the use of computer simulations in biological research, we are developing special-purpose simulators.

In this poster, we present a special-purpose simulator for intracellular calcium dynamics. This simulator does not require any programming-like skills for setting up, but does require inputs of several parameter values through a graphical user interface. In this simulator, a calcium-induced calcium release mechanism is modeled by Nagumo equation, a reaction-diffusion equation where its reaction term is modeled as a cubic polynomial. Numerical solutions are obtained by using explicit method. We are planning to develop a variety of special-purpose simulators so that biologists can find suitable ones for their research.

(COI: No)

P1-212

CellCompiler: Multiscale biological function model simulator which can use complex calculation schemes

Suzuki, Yohei[1,2,3]; Arita, Takeru[1]; Komiyama, Shigeru[1]; Punzalan, Florenciorusty[1]; Shimayoshi, Takao[2]; Kunieda, Yoshitoshi[3]; Amano, Akira[1] (*[1]College of Life Sciences, Ritsumeikan University, Shiga, Japan; [2]Graduate School of Informatics, Kyoto University, Kyoto, Japan; [3]College of Information Science and Engineering, Ritsumeikan University, Shiga, Japan*)

We have developed a code generator software called CellCompiler, which can automatically generate programs for biological function simulations. The code generation system requires three inputs, namely a CellML or PHML file describing a biological model, a TecML file describing various calculation schemes to discretize and solve the biological model, and a RelML file relating the CellML and TecML file. The biological model may be a combination of multiple models with different temporal or spatial scales. TecML uses recurrence relations to describe various solution schemes. In the case of multiple models with temporal scale variety, it can represent a combination of multiple temporal scale calculations. As an example, consider a coupled pharmacokinetic model coupled with a cellular electrophysiology model. The two models have different time scales, hours for the pharmacokinetic model and milliseconds for the cell model. For this, the code generator creates a double loop; an inner loop containing the excitation propagation of the cell model and an outer loop containing the drug absorption. The code generator also allows users to automatically perform parameter studies to analyze the effect of different model parameters to simulation results.

(COI: No)

P1-213

Simultaneous measurements of sound evoked electrical activities and calcium responses from deep brain regions by photometric patch electrode

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Intracellular calcium increase associated with neural activity is essential for supporting neural functions such as neural plasticity. It is therefore important to know how calcium changes to elucidate underlying mechanisms of calcium regulation. Signaling molecules are generally monitored by optical methods. However, application of two-photon microscopy in the deep brain tissue imaging is impossible due to light scattering. We overcame this problem by using photometric patch electrode (PME) recording system that utilizes a patch electrode as a light guide and is enabled us to excite and obtain fluorescence from a target neuron simultaneously with electrical recording. We measured calcium sensitive Oregon Green BAPTA-1 fluorescence signal simultaneously with the field current in response to sound stimulus from various auditory nuclei of young chicken; Field-L (avian auditory cortex), inferior colliculus (IC), and nucleus magnocellularis (NM, avian cochlear nucleus). We found distinct calcium fluorescence signals in ascending order across the nucleus from NM to Field-L. Neurons in NM practically suppressed calcium increase during orthodromic excitation. NM is a relay nucleus and receives inputs of high frequency activity of auditory nerve fibers and has high rate of spontaneous and driven firing activity; thus calcium influx is likely suppressed otherwise neurons are poisoned to apoptosis.

(COI: No)

P1-214

Quantification of the axonal transport activity of cultured neurons by flow analysis (II)

Katakura, Takashi; Isonaka, Risa; Kawakami, Tadashi (*Dept Pysiol, Kitasato Univ Sch Med, Kanagawa, Japan*)

We used KBI Flow Analysis plugin on Image J in our study. We defined previously the activity of axonal transport as a sum of particles which moves more than 0.3 pixels per frame (0.1 μm/s) within a limited area and a limited time. Our present study revealed that the greater part of the apparent velocity calculated by Flow analysis after the fixation of observed neurons by 4 % paraformaldehyde at the end of each experiment is less than 0.3 pixels per frame. The value less than 0.3 contains artificial errors derived from diffused reflection and such, which should be eliminated. The activity value based on our definition is well coincided with the value, Transported organelles (% of control) obtained from our traditional method. Another problem, where the velocity estimation of the particle sometimes fails because of the high speed of the moving particles, can be solved by applying an appropriate small value, 2 or 4 pixels but 8, to stepXy. We can also distinguish and sum up the number of anterograde transporting particles and retrograde transporting particles separately by an angle of calculated velocity vectors. The next step of our study is to write a series of macro programs in order to automate huge numbers of calculations.

(COI: No)

P1-215

Magnetic Resonance Imaging using human fetuses preserved in formalin solution

Kamatani, Mikako; Yamamoto, Akira; Miyazaki, Reina; Makishima, Haruyuki; Okada, Tomohisa; Togashi, Kaori; Yamada, Shigehito (*Kyoto Univ. Grad. Sch. Med., Kyoto, Japan*)

To understand the mechanism of development and disorder during childhood, the morphological changes during fetal period has been focused and recently some fetal brains have been imaged by magnetic resonance imaging (MRI). Those images were quite fine, however, obtained by isolated brain samples; there is no definitive imaging protocol for whole body samples. The Kyoto Collection is the world largest collection of human conceptuses, and contains almost 40,000 embryos and 5,500 fetuses, stored at the Congenital Anomaly Research Center, Kyoto University Graduate School of Medicine. The fetuses have been fixed and preserved in the formalin solution. To establish the protocol suitable for MRI using whole-body fetuses, here we imaged 12 fetuses using MRI system with 3T magnet (SIEMENS MAGNETOM SKYRA). For magnetization-prepared rapid gradient-echo (MP-RAGE) sequence, no treatment were not required for fetuses in formalin solution to obtain fine images; but less signals could be obtained from the specimens in T2-weighted and diffusion-weighted imaging (DWI). To increase the signals obtained from water, the fetuses were soaked in phosphate buffered saline for 3-14 days and the T2 and DWI signals were improved after the substitution. The specimens with long-term preservation in formalin solution were available for T2 and DWI as well as MP-RAGE, and it implies the possibility for diffusion tensor imaging (DTI) using abundant specimens from the Kyoto Collection.

(COI: No)

P1-216

Thiel's fixation method to prepare cadavers for surgical training

Doihara, Takuya[1]; Shimokawa, Tetsuya[1]; Nabeka, Hiroaki[1]; Kobayashi, Naoto[1]; Matsuda, Seiji[2] (*[1]Anat Embryol. Ehime Univ Grad Med., To-on, Japan; [2]Education C. Ehime Univ Grad Med., To-on, Japan*)

Following the release of "Guidelines for Cadaver Dissection in Education and Research of Clinical Medicine, " we began extensive surgical training at Ehime University in 2012. In addition, a surgical training center was established at Ehime University in December of 2013. The use of conventional 10% formalin-fixed cadavers for surgical training is unsuitable because their tissues are much harder than those of living bodies. Therefore, as a substitute for formalin when preparing cadavers for surgical training, we performed Thiel's fixation method. We purchased "Thiel's fixation method liquid" from A. S. CHEMICAL Co., Ltd. (Concord, ON, Japan) and added two types of blood, resolvent and formalin (3.9%), to it. We then fixed the cadavers by injecting the total solution via the femoral artery. Cadavers fixed using Thiel's method retained the softness of a living human body; these cadavers were useful for surgical training (e.g., laparoscopic surgery). However, there were some problems, including individual differences in the fixed state of the abdominal organs and occasional insufficient fixation of the brain. It is necessary to perform suitable cadaver fixation for the purpose of surgical training by adjusting the formalin content in the fixation liquid and improving the infusion method.

(COI: No)

P1-217

Anatomical observation of cadavers embalmed with 10% N-Vinyl-2-Pyrrolidone

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N-Vinyl-2-Pyrrolidone (NVP) solution was used for embalming cadavers as a substitution fixative of formalin (FA), as reported in the 116th Annual Meeting of the Japanese Association of Anatomists (Fujikura et al, 2011). Formerly, the effect of NVP as a fixative was studied histologically, using the animal tissue (Fujikura et al, 2008, 2009). At present, we are continuing observation of cadavers fixed with NVP of various concentrations to pursue an optimal condition. In the 119th Annual Meeting of the Japanese Association of Anatomists (2014, Tochigi), we reported the observations on cadavers embalmed with different NVP solutions: the final concentration in the tissue was 4.0, 4.2, 5.4, 5.5, 10.2, 10.5, and 21.5%. In that observation, the 4 cadavers containing lower concentration of NVP were very soft and vulnerable, but the ligaments were easily identified through the transparent connective tissue. On the contrary, the connective tissue was too hard and opacified in the cadaver fixed at the highest concentration of NVP, though the original shape of the organs was well preserved. These observations revealed that the cadavers became harder with increasing final tissue concentration of NVP, and that 10% NVP was suitable for dissection by students. In the present study, we report the observation of the cadavers fixed with 10% NVP, especially on the several joints, hearts and brains.

(COI: No)

P1-218

Usefulness of the cadavers embalmed by the saturated salt solution method for surgical training: The evaluation of surgeons and feasibilities of clinical procedures

Hayashi, Shogo[1]; Kawata, Shinichi[1]; Qu, Ning[1]; Hatayama, Naoyuki[1]; Naito, Munekazu[2]; Hirai, Shuichi[1]; Itoh, Masahiro[1] (*[1]Dept. Anat., Tokyo Med. Univ., Tokyo, Japan; [2]Dept. Anat., Aichi Med. Univ., Nagakute, Japan*)

Background: For surgical training (ST) courses using cadavers performed to advance a surgeon's techniques without any risk to patients, the new embalming methods to make cadavers the more soft and safe are desired. The aim of this study is to evaluate the suitability of cadavers embalmed by the saturated salt solution (SSS) method for ST.
Methods: Six cadavers were embalmed by three methods: formalin solution, Thiel's solution (TS), and SSS methods. Fourteen surgeons evaluated the three embalming methods. Furthermore, seven trauma surgeons and two orthopedists operated these cadavers by 21 procedures. In addition, ultrasonography, central venous catheterization, and incision with cauterization followed by autosuture stapling were performed.
Results: The surgeons evaluated the cadavers embalmed by the SSS method to be highly equal to those embalmed by the TS method. Ultrasound images were clear in the cadavers embalmed by both TS and SSS methods. Central venous catheterization could be performed in a cadaver embalmed by the SSS method and then be affirmed by X-ray. Lungs and intestines could be incised with cauterization and autosuture stapling in the cadavers embalmed by TS and SSS methods.
Conclusion: Cadavers embalmed by the SSS method are sufficiently useful for ST not less than ones embalmed by the TS method. The SSS method is considered to have a beneficial feaure that it is simple and low-cost.

(COI: No)

P1-219

Comparison of embalming methods from the aspect of suitability for surgical training: On their antiseptic effect and cadaver fixation

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Objective: To compare the cadavers embalmed by the several new embalming methods for surgical training from the aspect of their antiseptic effect and hardness of cadaver tissues.
Methods: Four cadavers were prepared by conventional formalin fixation method (formaldehyde: FA 3.7%), Thiel method (FA 1.8%), Preserve(R)fixation method (FA 0%), Saturated salt solution (SSS) method (FA 0.75%). Bacterial and fungal culture tests, dissection and histological observation by Hematoxylin-Eosin staining were performed.
Results: Each method performed much the same antibiotic effect immediately after injection. The FA embalmed cadaver seemed too rigid for surgical training. Thiel embalmed cadaver had the greatest joint ranges of motions. The internal organs of this cadaver were damaged gradually after opening of the body cavities. The hardness of the cadaver embalmed by Preserve(R)fixation showed the same tendency. The hardness of the cadaver embalmed by SSS method was intermediate.
Discussion: Although the Thiel embalmed cadaver is so close to a living body and suitable for a clinical training orientation, the improvement of ex-post treatment may be required for long-term use. Preserve(R)fixation method will be improved by adjustment of the composition, especially alcohol concentration. Although the SSS method is poorly understood, it may be relatively suitable for surgical training as it stands.

(COI: No)

P1-220

Quantitative imaging by a newly endoscopic system for pathological malignancy status based on endogenous fluorescence

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Background: We found that a nitrosamine-induced esophageal tumor model rat showed strong endogenous fluorescence, which was highly related with emergence of atypical cells, hyperplasia and tumorous changes in epithelia. Hence, the aim of this study was to clarify the origin of fluorescence and to quantitatively visualize the pathological status with the endogenous fluorescence as a clue using a fluorescence multi-spectral imaging (FMSI) system.
Methods and Results: We obtained fluorescence multi-spectral images of mucosal membrane of extracted esophagus from the rats that were administrated N-nitroso methyl butylamine(NMBA) (15 mg/L) in a drinking water for 1-16 weeks. The FMSI showed fluorescence with a peak of 630 nm (excitation: 405 nm) in areas where atypical cells exist, and intensity of the fluorescence was positively correlated with the time period of NMBA administration. HPLC revealed that an origin of the endogenous fluorescence was protoporphyrin IX and other porphyrins (e.g. uroproporphyrinogen) were not detected. For in-vivo realtime quantitative imaging, we developed an endoscope-based FMSI system equipped with spectral unmixing mechanism. The novel endoscopic system made it possible for us to detect early lesions by transesophageal approach.
Conclusion: The newly developed endoscope-based FMSI system can be a promising tool for the detection of precancerous lesions based on endogenous fluorescence.

(COI: No)

P1-221

Directly observed membrane disruption and resealing during centrifugation of sea urchin eggs by centrifuge polarizing microscope

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Large plasma membrane disruptions (PMDs) rapidly invoke a localized exocytotic reaction that adds a 'patch' of internal membrane to the plasma membrane at the PMD site, a calcium-dependent resealing mechanism. We have used sea urchin eggs as a model system to define the mechanistic basis of this fundamental cell survival response. Here we directly observed plasma membrane tears that occur in sea urchin eggs during centrifugation with a special centrifuge polarizing microscope (CPM). Dilute suspensions of unfertilized eggs were layered in a centrifuge chamber above an osmotically matched dense solution containing Percoll, forming a density gradient that allowed the eggs to slowly settle to an equilibrium position. Centrifugation at speeds of up to 8,000 rpm for 20 min, separated the eggs into two parts. One part was filled with yolk granules and internal vesicles, the second part was filled with clear cytoplasm. These membrane tears by shear forces did not show variously shaped surface projections involved in exocytosis at the PMDs. These cell separations depended on the presence of calcium. However, sea urchin eggs were broken by this centrifugation in the absence of calcium. The part filled with yolk granules and internal vesicles repaired the PMDs made by a two- photon laser, but the part of the eggs containing clear cytoplasm did not repair.

(COI: No)

P1-222

The 3D-atlas of adult zebrafish

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Zebrafish is an experimental model animal, which is used to study the development of the tissue and the body. Zebrafish embryos have clear bodies, and allow us to perform hole mount microscopy to know the 3-dimentional (3D) structures of the tissues and whole body. When zebrafishes grow up into adults, they are observed by sectioning and microscopy in general. Sections of adult zebrafish provide the 2-dimentional (2D) information, but lack the 3D-information of a tissue or a whole body. For the 3D analysis of adult zebrafishes, we utilized the serial sectional images, and reconstructed in a personal computer with a free softwere, OsiriX. XY resolution of the original 2D image is $4.7 \times 4.7\,\mu$m. XYZ resolution of reconstructed 3D image $18.8 \times 18.8 \times 20\,\mu$m. The image quality, including resolution, brightness and contrast was enough to observe various organs, for example brain, skeletal system, vasculature and gastrointestinal system. Zebrafishes are often used in the phenotype analysis after gene manipulations. Our atlas of adult zebrafish should be useful as the basic knowledge for such analysis.

(COI: No)

P1-223

Advances in open-skull surgery for *in vivo* imaging by biocompatible materials

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To understand the mechanisms of learning and memory, it is important to observe how neural circuits are activated, modified, and maintained in the living mouse brain. Two-photon microscopy is a useful tool for observing the neural circuits that extend across brain regions *in vivo*; this method can penetrate deep into thick specimens, achieve less invasive optical sectioning, and provide 3D images reconstructed from these sections. However, the quality of *in vivo* images is dependent on the transparency of the cranial window, which is in turn affected by the experimentalist's technical skill in the open-skull surgery that replaces the cranial bone with cover glass. In addition, even after a successful surgery, the cranial window tends to become cloudy several days later. Consequently, it becomes difficult to observe the neural circuits *in vivo* over long periods.
Here, we report new methods for keeping cranial windows clear using two biocompatible materials. The anti-thrombogenic biocompatible material Lipidure® kept the cranial window clear for long periods. Another biocompatible material, Cocktail X, increased the fluorescence signals emitted from neurons and allowed sharp visualization of fine structures at deep regions. These biocompatible materials should be useful for studies of changes in neural circuits at the synapse level over long periods.
(COI: No)

P1-224

Evaluation of *in vivo* two-photon microscopy by imaging of embedded fluorescent beads in mouse brain

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Morphological changes in post-synaptic structures (dendritic spines) are thought to be involved in synaptic plasticity, which is implicated in information processing by the neural network. Therefore, in order to understand brain functions, it is important to visualize synapses in the living mouse brain. Because of its high resolution and deep imaging capability, *in vivo* two-photon microscopy has been used to observe dendritic spines under live conditions. In our previous study, we found that penetration depth could be improved by changing the diameter of the irradiation excitation laser. However, the resolution under these conditions was not determined, because the details of the focal spot size of the excitation light were not measured precisely. In general, the resolution of a laser scanning microscope is reversibly correlated with the focal spot size. This size is sometimes evaluated by measuring full width at half maximum (FWHM) of a structure with a known shape (e.g., a fluorescent bead) that is smaller than the diffraction limit. In this study, we injected fluorescent beads into the living mouse brain, and succeeded in *in vivo* two-photon imaging of single beads at various depths in the brain. We estimated the resolutions by measuring FWHM from single-bead images.
(COI: No)

P1-225

Direct measurement of the binding rate constant of kinesin to microtubules in living cells

Kambara, Taketoshi; Okada, Yasushi (*QBiC, RIKEN, Osaka, Japan*)

It has been established that conventional kinesin (kinesin-1, KIF5 in mammalian cells) selectively moves along a specific subset of microtubules in living cells. For example, KIF5 is specifically recruited to the microtubules in the axon initial segment in neurons, which would enable efficient transport into the axon. However, the mechanism of this selective binding is still controversial. Some groups have proposed that acetylation or other post translational modifications of tubulin serve as the cue for selective binding. We are proposing that conformational differences between the GTP-form and GDP-form of microtubules provide the cue. To test this idea, it would be important to examine whether kinesin binding to specific subsets of microtubules is enhanced, inhibited or both. Here, we measured the binding rate constant of kinesin to microtubules in living cells and in vitro using single molecule fluorescence microscopy. To our surprise, the binding rate constant of KIF5 to the track-microtubule in vivo was nearly ten times higher than that in vitro, suggesting that mechanisms exist in the cell to recruit KIF5 specifically to some subset of microtubules by accelerating the binding reaction.
(COI: No)

P1-226

Quantitative measurement of ATP concentration inside single mammalian cells

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Despite the fact that adenosine triphosphate (ATP) is required for a wide variety of intracellular processes, it is not clear how the synthesis and consumption of ATP is balanced inside living mammalian cells. Since the energy required at each subcellular domains or organelles could vary from site to site or change over time, subcellular localization and fluctuation of ATP concentration are important. We previously developed a new fluorescent ATP indicator protein named "QUEEN" that can be used to quantitatively measure absolute ATP concentrations inside living bacterial cells. Here, we developed an improved version of QUEEN suitable for application in mammalian culture cells. We expressed this indicator in mammalian cultured cells and successfully measured the ATP concentration inside different organelles. In addition, we have also examined the time-dependent change of ATP concentration in response to exogenous perturbations.
(COI: No)

P1-227

High-resolution imaging of live cells and tissues by scanning ion conductance microscopy

Nakajima, Masato; Ushiki, Tatsuo (*Dep. Microsc. Anat., Niigata Univ. Grad. Sch. Med. Dent. Sci, Niigata, Japan*)

Scanning ion conductance microscopy (SICM), introduced by Hansma in 1989, is a technique of scanning probe microscopy and uses a microglass pipette as a sensitive probe. Because it can obtain contact-free images of the sample topography, SICM is expected to be used for studying the surface structure of soft biological samples under liquid conditions. We previously showed that hopping mode SICM is useful for imaging complicated surface structures of fixed cells and tissues in liquid conditions. The present study was performed for the assessment of the usefulness of SICM for observing biological samples without fixation. We succeeded in obtaining SICM images of live cultivated cells without any severe sample damages, which might be caused by the probing tip. The minimum data acquisition time per image (128 by 128 pixels) was about 8～10min. The movement of cellular processes were clearly seen on the surface or the periphery of the cells, indicating that SICM is useful not only for observing fixed cells, but also for analyzing the movement of live cells. In this study, we will also show the applicability of SICM to the study of the surface structure of live tissue samples (e.g., the epithelial surface of the urinary bladder).
(COI: No)

P1-228

Application of Sihler's staining in histology

Hayashi, Hiroyuki[1]; Kimura, Akihiko[2]; Kanemura, Naohiko[1]; Nishihara, Ken[1]; Gomi, Toshiaki[2]; Naruse, Hideo[2] (*[1]Saitama Prefec. Univ., Saitama, Japan; [2]Tokyo Ariake Univ., Tokyo, Japan*)

Sihler's staining is a technique for staining nerve endings in muscle. This technique involves decalcification of the specimen and it renders soft tissue transparent. This study was conducted to determine if this staining could be used to study both the macroscopic anatomy and histology of the same specimens. An experiment was performed using ICR mice. The femoral region was separated starting at the hip joint. Specimen was fixed in 10% neutral buffered formalin. The specimen was then rinsed with water and subjected to Sihler's staining. The specimen was stored in 100% glycerol. Nerves were studied macroscopically and photographed. Afterwards, glycerol was washed off with water and specimens were embedded in paraffin. Specimens were sectioned, stained with eosin, and then examined using microscopy. Sihler's staining resulted in hematoxylin staining of nerve fibers innervating the leg, facilitating their macroscopic observation. After nerves were examined macroscopically, tissue sections were prepared. The result was a staining technique that did not require a special decalcification step and that allowed ready sectioning of bone. However, the hematoxylin staining of nerve fibers that were verified macroscopically faded, precluding a clear depiction of their histology. This was because the staining of nerve fibers faded during the preparation of tissue specimens. A remedy to this problem will result in Sihler's staining being a useful staining technique to study both the macroscopic anatomy and histology of the same specimens.
(COI: No)

P1-229

Real time measurement of pharmacokinetics of an ototoxic drug in the microspace of in vivo inner ear by a diamond microelectrode

Ogata, Genki[1,2]; Nin, Fumiaki[1,2]; Ishii, Yuya[3]; Asai, Kai[3]; Yoshida, Takamasa[1,4]; Einaga, Yasuaki[3]; Hibino, Hiroshi[1,2] (*[1]Dept Mol Physiol, Niigata Univ Med Sch. Niigata, Japan; [2]Ctr for Transdisciplinary Res, Niigata Univ, Niigata, Japan; [3]Dept of Chem, Faculty of Sci and Tech, Keio Univ, Tokyo, Japan; [4]Dept Otolaryngol, Grad Sch Med, Kyushu Univ, Fukuoka, Japan*)

A loop diuretic, bumetanide, often damages inner ear and causes dizziness and deafness. To develop the therapeutic strategies that reduce such side effects and design the analogs nontoxic for hearing, real-time monitoring of the pharmacokinetics local environments of in vivo inner ear is necessary. Several methods are available to determine the concentrations of bumetanide in body fluids such as HPLC, and mass spectrometry. These measurements require large amounts of samples taken from body, and thereby enable to detect the dynamics in the microspace continuously. Also, some of them represent a low sensitivity. To resolve these problems, we utilized boron doped diamond (BDD) microelectrode. The electrode was inserted into the inner ear fluid of living guinea pig. On the chronoamperometry, we successfully observed increase of the oxidation current elicited by bumetanide in several seconds after injecting the drug of 30 mg intravenously. Calibration curve demonstrated that the peak current approximately corresponded to 532 μg/dl. Converse response was detected in an inner-ears potential that mirrors hearing level and was measured by a glass microelectrode. Our BDD microelectrode system can be applied to monitor other drugs in numerous tissues and organs and helpful to promote the pharmacological researches.

(COI: No)

P1-230

The development of PET imaging for detecting AMPA receptors trafficking during acquisition of fear memory

Shibata, Yusuke; Serizawa, Asami; Kuroki, Yoko; Miyazaki, Tomoyuki; Takahashi, Takuya (*Dept Physiol, Grad Sch Med, Yokohama city Univ, Yokohama, Japan*)

When experiencing or learning, some plastic changes occur in synapses and drive AMPA receptors (AMPA-Rs) into postsynaptic membrane. Moreover, interruption of AMPA-Rs trafficking into synapse disrupts newly learning. These studies establish that AMPA-Rs work as a key molecular machinery underlying experience and learning. Recent studies using postmortem brains revealed the quantitative alteration of AMPA-Rs may relate to some variety of mental disorders including schizophrenia, depression and ASD. Among these studies, membrane numbers of AMPA-Rs seem to account for these disorders. However, with the current techniques, we cannot observe the behavior of AMPA-Rs in living human brains. Our study aim to develop new PET probes to detect the membrane numbers of AMPA-Rs in living human brains. We disclosed that animals experiencing inhibitory avoidance (IA) task increase the membrane surface expressions of AMPA-Rs up to 1.5-fold of control animals in the hippocampus. To detect these changes in vivo, we developed new method using LC/MS-MS. Briefly, we administered compounds previously known to bind to AMPA-Rs specifically to adult rats intravenously. 24 hours later, we dissected hippocampus and measure these compounds in the hippocampus using LC/MS-MS. Then we analyze the correlation between biochemical data and MS data. As a result, some compounds increased in IA+ animals compared to control animals. These results indicate that these compounds detect the increase of surface expression of AMPA-Rs in vivo.

(COI: No)

P1-231

A new method to isolate basophils from peripheral blood without dilution or hemolysis by the flow-through density gradient centrifugation

Shiono, Hiroyuki[1]; Matsui, Takuya[1]; Masubuchi, Satoru[1]; Ito, Yoichiro[2] (*[1]Dept Physiol, Aichi Med Univ Sch Med, Aichi, Japan; [2]Lab Biosep Tech, Biochem Biophysics Cent, NIH, Bethesda, MD, USA*)

We have developed a novel flow-through density gradient cell separation method. This system continuously separates a large number of cells into five fractions according to their densities. As the blood contains a huge number of red blood cells (over 1,000 times of the number of leukocytes), the pretreatment with dilution and hemolysis is usually essential for harvesting leukocytes. However, we separated basophils, which had the fewest number among leukocyte population, from 20ml of human peripheral blood in 3 hours without the pretreatment. A set of isosmotic Percoll media with the densities of 1.050, 1.074, 1.079, 1.090, 1.095 and 1.104g/ml was prepared, and introduced into the channel to form a density gradient. Then the anti-coagulated blood was continuously fed into inlet 1, through which Percoll medium with the density of 1.050g/ml was flowing. Harvested fractions with the density of 1.079g/ml and 1.090g/ml were washed, and the cell pellets were re-suspended into 1ml of the density medium with 1.050g/ml. This cell suspension was fed through inlet 1 and separated under the same condition, again. Separated cells in the density of 1.079g/ml and 1.090 g/ml by the second run contained basophils at about 72% and neutrophils at about 92%, respectively. The red blood cell counts were about 6% in each fraction. Without diluting the blood sample shortened the time required for cell separation and the repeating operation made possible to remove most of red blood cells.

(COI: No)

P1-232

Novel mouse xenograft model for noninvasive *in vivo* imaging of human tumor cell and tissue in the auricle

Kita, Sayaka; Higuchi, Hideo (*Grad. Sch. Sci., The Univ. of Tokyo, Tokyo, Japan*)

We developed methods of preparing xenograft model and imaging GFP-expressing cells to observe noninvasively cells in mouse auricles. We selected the ear auricle of mouse for observation of tumor cells because of very thin (about 150-200 μm) and limited hypodermal tissue. We have developed a novel xenograft model which has tumor in auricle. We injected five kinds of human cancer cell lines into the ear auricle of SCID mice; breast cancer cell lines named KPL4-EB1-GFP and MDA-MB-231, MDA-MB-231-EB1-GFP and MDA-MB-231-GFP-tub, and glioma cells line U87MG. Tumor was successfully formed 100% of injected mice at incitation of $> 4.6 \times 10^6$ cells in all cell lines. The tumor and cells in auricle were noninvasively imaged by spinning disk confocal (CSU) system equipped with automatic positioning stage, piezo actuator for objective and an EMCCD camera. We imaged GFP fluorescence in the MDA-MB-321-GFP-tub cells in tumor of ear auricle without injuring mice. The individual two cells in tumor were distinguished faintly with bright background of tumor fluorescence. We also took a montage view of tumor cover wide area (3 × 2 mm). The shape of a tumor appeared faintly at the depth > 40 μm, suggesting the shape is background of a tumor located deeper. There are several bright spots in the diameter of about 20 μm in the enlarged image, indicating those are single cells. We could successfully perform real time observation of GFP fluorescence in the breast cancer cells in noninvasive condition by a CSU system.

(COI: No)

P1-233

Thermo stabilized super-active TALEN mediated highly efficient and homogeneous gene knock-out in mammalian embryos

Ikeda, Kazuho; Terahara, Yoko; Sumiyama, Kenta; Okada, Yasushi (*RIKEN, Osaka, Japan*)

Gene editing *in vivo* has become possible by the development of artificial nucleases that can be designed to cut the genome DNA selectively at the target site in the genome. TALENs are highly specific artificial nucleases, has been proven to be useful for the genome editing in lower vertebrates such as zebrafish and *Xenopus*. However in mammalian cells and embryos, TALENs often show poor activity, which limited its applications. To overcome this limitation of TALEN, we introduced amino acid substitutions into TALE DNA binding domain which might participate in conformational stability under high temperature. Several mutations were introduced, and some successfully showed significantly higher activity at 37 °C both *in vitro* and *in vivo*. TALENs made from this high activity mutant (named "super-active" TALEN) showed significantly higher rate of genome editing in zebrafish eggs. Finally we have demonstrated that our super-active TALEN efficiently introduced site specific mutations in mouse embryos. Interestingly, genome analyses detected only less than four different mutant alleles in each baby. In the case of CRISPR/Cas9 mediated gene editing, however, more than eight alleles were often detected. These results suggest that super-active TALEN shows its activity at two-cell stage, earlier than CRISPR/Cas9, thus super-active TALEN might serve as an effective tool for the genome editing in mammalian cells and embryos.

(COI: No)

P1-234

In vitro analysis of thermo stabilized super-active TALEN

Terahara, Yoko; Ikeda, Kazuho; Miyashita, Naoyuki; Okada, Yasushi (*Quantitative Biology Center, RIKEN, Osaka, Japan*)

Gene editing *in vivo* has become possible by the development of artificial nucleases that can be designed to cut the genome DNA selectively at the target site in the genome. TALENs are highly specific artificial nucleases, has been proven to be useful for the genome editing in lower vertebrates such as zebrafish and Xenopus. However in mammalian cells and embryos, TALENs often show poor activity, which limited its applications. Recently we have developed "super-active" TALEN by introducing amino acid substitutions into specific residues of TALE DNA binding domain, and demonstrated that super-active TALEN can mediate efficient genome editing in mouse embryos and zebrafish eggs.

To evaluate how our "super-active" mutations actually affect enzymatic properties, we have produced active TALEN proteins in *E. Coli* expression system and measured their activities *in vitro*. Our data suggested that super-active TALEN is more stable at 37 °C, which might cause high activity. In addition, all-atom molecular dynamics simulations confirmed the stabilization of the conformation. The mutated residues apparently suppressed the intramolecular fluctuations. Our *in vitro* and *in silico* based approaches provide us a new insight for further improvement of TALEN techniques, including even more active TALEN, other gene editing enzymes, and new biological tools such as sequence specific DNA binding probes.

(COI: No)

P1-235

On the relationship of the distributions of the cutaneous nerves between the musculocutaneous nerve and the radial nerve

Saitou, Midori[1]; Yoshihara, Miku[1]; Tokita, Kounosuke[1,2]; Kojima, Ryuhei[1]; Aizawa, Yukio[2]; Kumaki, Katsuji[2]; Kageyama, Ikuo[2] (*[1]Phys. Th. Sch. Saitama. Med. Univ., Sitama, Japan; [2]Sch. Life dentistry at Niigata Nippon Dent. Univ., Niigata, Japan*)

It has been explained in many textbooks that the musculocutaneous nerve is the ventral nerve and the radial nerve is the dorsal nerve. However, we encountered a special case of the total defect of the superficial branch of the radial nerve, which was compensated by the lateral antebrachial cutaneous nerve from the musculocutaneous nerve. Now we show the details of the distribution of the cutaneous nerves in the hand region and the formation of the brachial plexus of this case. To compare with the normal state, we also examined the distributions of the cutaneous nerves in the hand in some normal cases. On the consequence, the lateral antebrachial cutaneous nerve generally communicated with superficial branch of the radial nerve at the radial side of the distal part of the forearm and distributed in some area of the dorsal side of the wrist region, while the radial nerve distributed into the palmar side of the thumb. Thus, the fibers in the musculocutaneous nerve was not only the ventral components and the radial nerve also contained not only the dorsal components. These indicated that the peripheral nerves should be considered as the road to the fibers, therefore, the cutaneous fibers to the dorsal hand could run in the musculocutaneos nerve substituted for the radial nerve.

(COI: No)

P1-236

Transposition of innervation to brachial flexors: musculocutaneus nerve variations

Shionoya, Kento[1]; Hayashi, Mari[1]; Hayashi, Shogo[2]; Qu, Ning[2]; Hatayama, Naoyuki[2]; Kawata, Shinichi[2]; Itoh, Masahiro[2] (*[1]Sch. Med. Tokyo. Med. Univ., Tokyo, Japan; [2]Depart. of Anat. Tokyo. Med. Univ., Tokyo, Japan*)

Backgrounds: A musculocutaneous nerve (MCN) generally innervates the brachial flexors (BF), i.e. the coracobrachialis (CB), biceps brachii (BB) and brachialis (B). As variations of MCN, the absence of MCN and the communicating branch (Com) with median nerve (MN) are known. Furthermore, the cases that MN innervates to some BF have been also reported as the translocation of BF innervations.
Methods: Sixty two upper limbs were observed. In each limb, the variations of MCN and the nerves innervating the BF were recorded.
Results: In all cases, the BF branches came out in the order of the CB, BB, and B. Sixteen limbs had the Com between the MCN and MN. The absence of MCN was observed in one limb. Translocation of the BF innervations was observed in one limb. In this case and all previously reported cases of the translocation, the Com between MCN and MN were observed. Furthermore, in all cases of the translocations, no BF branch arose from the MCN distal to the Com.
Discussion: Depending on these characteristics of the BF branches and the origins of the Com, MCN variations could be classified theoretically into five patterns. Although all patterns were not covered in the present upper limbs, these patterns were covered including the previous reported cases without exception.
Conclusion: These results suggest that the translocation of BF innervations closely relates to the communication between MCN and MN.

(COI: No)

P1-237

Sex difference in the location of the obturator nerve leaving pelvic cavity

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Background: To prospect location of the obturator nerve (ON) from the body surface is difficult. The aim of this study is to evaluate the location of the ON and its sex difference.
Methods: Twenty-seven bisected pelvises of human cadavers (10 males and 17 females) were observed. After the external obturator muscle was exhibited on the pelvic surface, the following measurements were carried out; the distance between pubic tubercle and the ON (D1); the angles between the inguinal ligament and the ON, at pubic tubercle (A1); the minimum distance between the ON and the inguinal ligament (D2); and the angles formed by inguinal ligament and a line which links pubic tubercle and ischial tuberosity (A2).
Results: The ONs were located on anterolateral part of obturator foramen in all cases. D1s were partially correlated with D2s ($r=0.45$, $p=0.017$). The significant sex differences in A1s (94.0 ± 3.36 degree in males and 86.4 ± 4.08 degree in females, $p<0.0001$) and A2s (106.6 ± 5.37 degree in males and 95.3 ± 3.47 degree in females, $p<0.0001$) were detected.
Conclusion: In females, ONs tended to leave pelvises more smaller angle than those of males. This difference may reflect sex difference in the form of pelvis.

(COI: No)

P1-238

Constantly existence of the sensory branch in the nerve to the pyramidalis muscle and gender difference of the course in the nerve

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The pyramidalis (Py) is often absent in human-being. It is very important to investigate distribution patterns of the nerve to the Py to study morphogenesis of its muscle. Sensory branches from the nerve to the Py were constantly observed. Ten cases out of six cadavers were studied. In all cases, the sensory branches from the nerve to the Py innervated to the tendon of Py, the rectus abdominis, internal oblique and the linea alba were found. Additionally, gender difference in the course of the ilioinguinal and genital branch of the genitofemoral nerves was observed. The nerve to the Py appeared from the superficial inguinal ring went downward and upward immediately in male cases. Namely, it made U-shaped course in male. However, the course of the nerve to the Py in females was straight. We suggest the U-shaped course of the nerves was based on the results of the descensus testis. It is important to investigate the distribution of sensory branches and gender differences on the course of the nerve to the Py in the absent case of the Py.

(COI: No)

P1-239

Anatomical variations of arterial supply to the spleen in rabbits

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The morphology of the spleen differs depending on each species. The rabbit that is widely used as an experimental animal is popular recently among companion animals. The rabbit has the flat, elongated spleen with the longitudinal hilus running along the visceral surface. However, the arterial distribution to the spleen has not been studied in detail so far. Thus, we studied anatomical variations of arterial supply to the rabbit spleen. Twenty-six male and 5 female New Zealand White rabbits, weighting 2.5-3.0 kg, were used. In the cadaver fixed with formalin, a cannula was inserted into the thoracic aorta to be injected with colored latex. After further fixation for more than 7 days, we observed the macroscopic arterial supply to the spleen. The splenic artery arises as the first independent branch of the celiac artery, and runs toward the splenic hilus to provide the splenic branches, whose number is predominantly 5 or 6, to the paremchyma of the spleen. The splenic branch often arises as a trunk (predominantly 1 ranging from 0 to 4) in common with the short gastric arteries (predominantly 4 ranging from 3 to 6) that distribute to the greater curvature or the visceral surface of the stomach. The terminal branch of the splenic artery continues as the left gastroepiploic artery toward the greater curvature. The results demonstrate that the pattern of arterial distributions to the spleen varies depending on each specimen, suggesting that such variations must be considered at experimental and veterinary surgical treatments in rabbits.

(COI: No)

P1-240

Distribution of artery in the maxillary sinus

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A dental implant is the advanced dental care at present, and is developed new methods. When especially the bone resorption in an upper molar part is remarkable, the maxillary sinus (MS) floor augmentation which increases the thickness of a bone required for an implant operation is performed. However, the detailed study of the distribution of artery in the maxillary sinus was not performed.
Methods: We used 13 maxillary sinuses in 42 cadavers which had the thickness of bone at the upper molar area. Then the height of the artery/groove from the bottom of MS and thickness of the artery were measured and then the branches from the infraorbital artery were dissected.
Results and Discussion: The height of the blood vessel from the bottom of MS runs to anteroposterior direction among blood vessels macroscopically observable as a blood vessel or it's groove on the lateral wall of MS was 6.2mm of average. The artery running anteroposterior direction on the lateral wall of MS was formed Posterior superior alveolar artery from a posterior wall and the anterior superior alveolar artery from an anterior wall as the past report. Additionally, we checked that the median superior alveolar artery (The name of his artery is not describe in the textbook.) and malar artery (The name of this artery is not described in human anatomical textbook.). From the above results, we suggest naming it the "intrasinual (arterial) arch".

(COI: No)

P1-241

A rare case report of inferior vena cava duplication

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The inferior vena cava (IVC) is the largest vein in the body, and functionally important for the majority of venous return from the lower extremities and abdomen, and drains into the right atrium. The double IVC or duplication of IVC is a well-known congenital anomaly and the incidence has been reported to be 0.2%-3%. Most duplicated IVC cases are clinically asymptomatic. However, since it has been reported that duplicated IVCs tend to be found in patients with hydroneprosis and various other conditions, they may have significant clinical implications, such as deep venous thrombosis and unexpected hemorrhage. Here, we report a case of an anatomic variant of IVC duplication that was found in the cadaver of an 81-year-old Japanese female during a student dissection course at Aichi Gakuin University School of Dentistry. We will describe the detailed anatomical features of this case, and will also discuss about this from the developmental aspect, since IVC duplication is caused by abnormal connections and regressions of subcardinal veins during embryonic development of the IVC. The authors have no conflicts of interest to declare.

(COI: No)

P1-242

The continuity of the marginal artery system distributing the stomach and duodenum

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The gastric arteries (GA), the gastro-omental arteries (GOA) and the pancreaticoduodenal arteries (PDA) run along the wall of stomach and duodenum to give off terminal branches (Vasae rectae) supplying them. The situation is similar to the outermost arcades of jejunal / ileal arteries and the marginal artery of the colon. We hypothesize that they constitute a continual marginal artery system in the abdominal gastrointestinal tract. The aim of this study is to examine the continuity of the marginal arteries in the stomach and duodenum region. 5 human abdominal gastrointestinal tracts were investigated. The marginal arteries sent off series of the terminal branches to a longitudinal band-like area on the wall of stomach and duodenum. The longitudinal band supplied from GOA was situated along the greater curvature of stomach, and continued to the band on the anterior surface of duodenum supplied by the anterior PDA, which continued to the band on the jejunal wall. The other band of stomach supplied by GA was located along the lesser curvature. The distributing area of the supraduodenal arteries connected the band on lesser curvature and the band on posterior duodenal wall supplied by the posterior PDA. The posterior duodenal band terminated at the Treitz ligament. These result suggested that the gastroduodenal artery and its terminal branches, the supraduodenal arteries, constitute the marginal artery system of the duodenum. The marginal artery system is thought to be formed as double stream until the Treitz ligament.

(COI: No)

P1-243

A case of duplicated inferior vena cava

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A case of duplicated inferior vena cava (IVC) along with other anatomical vessel variations in a female cadaver is reported.
Duplicated inferior vena cava (D-IVC) is congenital venous anomaly has an incidence rate ranging from 0.3 % to 3 %. According to Adachi (1940), it has an incidence of 1.4 ± 0.34 %. More recently, diagnostic imaging has revealed that D-IVC tends to be found in patients with hydronephrosis and various other conditions. The clinical importance of this anomaly lies in three principal areas: the potential for misdiagnosis on imaging; technical difficulties during retroperitoneal surgery and their significance in relation to the etiology and management of venous thromboembolism. In this anomaly, the right and left iliac veins drain into ipsilateral vena cavas that ascend on either side of the abdominal aorta until they form a confluence at the level of the renal veins. During the anatomical practice at Saitama Medical University in 2014, a case of D-IVC was observed in a 93-year-old Japanese female cadaver. Bilateral inferior vena cava, left sided IVC receiving a left renal vein, and transiliac vein were found through dissection. In this study, we will discuss the literature with respect to embryological studies, morphological classifications, and disease correlations.

(COI: No)

P1-244

Observation of the lenticulostriate arteries

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The importance of the lenticulostriate arteries (LSA) was pointed out because the arteries distributed to the internal capsule, caudate and lentiform nucleus. Recently, with the development of 7.0-T MRI (although it has not been spread) the imaging of the arteries has been reported (Kang et al, 2009). We think, however, the observation results with the gross anatomy method on the arteries are very necessary, because it is the basic data of the arteries and could to offer consultations for the MRI imaging. For the purpose we investigated the LSA with twenty-four Japanese brain specimens (fixed with 15% formalin and preserved in 50% alcohol for 6 months), to report the branch number, branch pattern, the distance between the confluence point of the internal carotid artery with the posterior communicating artery to the origin point of the LSA.

(COI: No)

P1-245

Thoracic insufficiency syndrome in an elderly woman

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During the demonstration of an anatomical dissection for medical students at Tottori University, a narrow left thoracic cavity and flattened left lung was observed in an elderly woman who had died of pneumonia. Autopsy findings also included left scoliosis and deformity of the upper four ribs on the left side. These findings in the thorax and the left lung suggested that this patient had thoracic insufficiency syndrome (TIS), which is characterized by the inability of the thorax to support normal respiration or lung growth. Further histological examination of the lung tissues showed an absence of alveoli in the left lung. Therefore, the hypoplastic left lung was thought to have had negligible respiratory function. This suggests that hypoplastic lung tissue in TIS does not recover throughout life without appropriate treatment. The right lung, however, showed compensatory enlargement and had well-developed alveoli, although filled with inflammatory cells. Although the right lung had provided adequate respiratory function over the lifetime of the patient, pneumonia in the one normally functioning lung proved fatal in this elderly woman. This finding suggests that early surgical treatment is desirable to promote development of the thorax and lung on the affected side. Medical professionals should be aware of the potentially fatal consequences of pulmonary infections in limited functioning lung tissue in elderly TIS patients.

(COI: No)

P1-246

Two atypical cases of vertebral arteries

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In general, the vertebral arteries arise from the subclavian arteries, and enter the transverse foramens of the sixth cervical vertebra and pass through the transverse foramen of C1 (the atlas). They travel medially and posteriorly along the posterior arch of C1, penetrate the dura mater and enter the foramen magnum. We encountered two cases of vertebral arteries with anomalous origins, and courses in a Japanese 93-year-old (case 1) and a 101-year-old (case 2) Japanese female cadavers during the student dissection practice at Wakayama Medical University at 2012. In case 1, the left vertebral artery directly branched from the aortic arch and entered the left transverse foramen of the fourth cervical vertebra. The right vertebral artery branched from the right subclavian artery and entered the right transverse foramen of the fourth cervical vertebra. In case 2, the left vertebral artery ran medially into the vertebral canal immediately though the transverse foramen of the axis instead of passing through that of the atlas (a C2 segmental type of vertebral artery). The vertebral artery on the right side and the cervical nerves on both sides were in a conventional position.

(COI: No)

P1-247

The relationship between a maxillary sinus and superior alveolar nerves and vessels demonstrated by cone-beam CT combined with μ-CT and histological analyses

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Background: There is no available detailed data on three-dimensional courses of human superior alveolar nerves and vessels. This study aimed to clarify the relationship between a maxillary sinus and superior alveolar nerves and vessels using cone-beam computed tomography (CT) combined with μ-CT and histological analyses.
Methods: DICOM data obtained from the scanned heads/maxillae of cadavers for undergraduate dissection practice and skulls using cone-beam CT (MercuRay; Hitachi) were reconstructed into the three-dimensional (3D) images using a software (INTAGE Realia; KGT). The 3D images were compared with μ-CT (Elescan; Nittetsu Elex) images and histological sections.
Results: Cone-beam CT clarified the relationship between a maxillary sinus and the superior alveolar canals/grooves. The main anterior superior alveolar canal/groove ran anteriorly through the upper part of sinus, and terminated at the lower part of piriform aperture. The main posterior one ran through the lateral lower part of sinus and communicated with the anterior one. Histological analysis demonstrated the existence of nerves and vessels in these canals/grooves.
Conclusions: The cone-beam CT is suggestive to be the useful method to clarify the superior alveolar canals/grooves including nerves and vessels at the level of histological section.
(COI: No)

P1-248

Development of the skeletal model with facial and masticatory muscles to reproduce a three-dimensional positional relationship between these muscles

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Objectives: This study aimed to dissect the precise courses of facial and masticatory muscles and develop the skeletal model with muscles to reproduce a three-dimensional positional relationship between these muscles.
Methods: During the anatomical dissection courses held in the Nippon Dental University at Niigata and Niigata University, we investigated a three-dimension positional relationship between facial and masticatory muscles using human cadavers. Based on these findings, we made the prototype of facial and masticatory muscles using epoxy putty.
Results: There were variations in the courses of facial muscles between cadavers and even in the same cadaver. Some muscles changed their courses depending on distributions of blood vessels. Finally, we succeeded to make the prototype of facial and masticatory muscles to reproduce a three-dimensional positional relationship between these muscles based on anatomical knowledge.
Conclusions: Although there was moderate regularity in the regional distribution of facial muscles, different facial muscles compensated their spread each other, resulting in variations in the courses of facial muscles. The skeletal model with facial and masticatory muscles based on this knowledge is useful to understand a three-dimensional positional relationship between these muscles.
(COI: No)

P1-249

Comparative anatomical analysis of the itch neural circuit in mammals

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Recently, the spinal gastrin-releasing peptide (GRP)-receptor has been identified as an itch-specific mediator in the somatosensory system. We focused on GRP as a marker of itch neural circuit and demonstrated the expression of GRP in the small-sized dorsal root ganglion and trigeminal ganglion (TG) neurons, and axon terminals in the superficial layers of the spinal dorsal horn and trigeminal sensory nucleus caudalis (Vc) in male rats. In order to compare the GRP distribution in different mammalian species, we used male mouse (rodent), male suncus (insectivore), and male monkey (primate) by staining with toluidine blue and immunohistochemistry. Morphometric analysis showed that GRP was expressed in 7% of mouse, 12% of rat, and 9% of suncus TG neuron. GRP was found in small-sized TG neurons in mouse, rat, and monkey but in various types of suncus. GRP terminated the superficial layers of the spinal dorsal horn and Vc in mouse, rat, suncus, and monkey. These findings indicated that GRP is common mediator to mammalian sensory neurons.
(COI: No)

P1-250

Comparative study of the innervation pattern to the plantaris muscle between human and non-human primates

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In phylogenetically, the plantaris muscle (PM) constantly exists in non-human primates, though this muscle often lacks in human. To elucidate its phylogeny, it might be useful to examine and compare detailed innervation patterns in the human and non-human primates. We compared innervation pattern of the PM in specimens of human (6 sides; 1 side of these lacked the PM), chimpanzee (2 sides) and rhesus monkey (1 side). Epineurium of the tibial nerve was peeled in all specimens. In the rhesus monkey and chimpanzees, the nerve to the PM (NP) formed a common trunk with the nerve to the flexor digitorum fibularis muscle (NF). But, in one chimpanzee, an additional muscular branch to the soleus was found (NS2), which formed a common trunk with the NP. In all human cases, the branch equivalent to the NS2 of the chimpanzee existed and formed a common trunk with the NP. The NP forming the common trunk with the nerve to the gastrocnemius (NG) and soleus (NS) was found only in one human case. In the case in which the PM lacked, the NS2 formed the common trunk with the NG and NS in addition to the NF. These results suggest that in the human case in which the PM is lacking, muscular component of the PM might be mingled with the bipennate muscle part of the soleus innervated NS2 in the human to adapt increasing antigravitational activity during the evolutionary change.
(COI: No)

P1-251

Comparative anatomy of the teres major muscle in a rough-toothed dolphin (*Steno bredanensis*)

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In most textbooks (Romer & Parsons 1977; Stark 1982), the teres major muscle (tm) in mammals is described to be differentiated from the latissimus dorsi muscle (ld). However, Koizumi (2012, 2013) has clarified that the tm or its relevant muscles in monotremes and monitors had a close relationship with the subscapularis muscle (sb), not with the ld. This fact was confirmed in humans (Kato 1989). On the other hand, the tm of dolphins has been reported to receive the branch of the thoracodorsal nerve that innervated the ld. (Sekiya 2011; Takakura 1997). Therefore, in this study we have clarified the innervation of the tm in both arms of a rough-toothed dolphin. Results: the scapular spine and infraspinous fossa cannot be distinguished from each other and formed a flat surface. From the caudal one-third of this surface the tm was originated and inserted into the humerus joining with the ld. The thoracodorsal nerve and the several subscapular branches were branched off from the posterior cord, formed by the ventral rami of the lowest four cervical nerves (C5-8) and the first thoracic nerve (Th1). The tm was supplied by two different nerves. One was from the subscapular branches and the other from the thoracodorsal nerve. Observing the intramuscular distribution, the subscapular branches distributed into the cranial two-thirds of the tm and the branch from the thoracodorsal nerve supplied the caudal one-third of the tm. Based on this observations we will discuss the dual origin of the tm and the relationship among tm, ld and sb in dolphins.
(COI: No)

P1-252

Effects of decrine of mechanical stress on structure of tibial articular cartilage and growth plate in rats

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Purpose: Articular cartilage and growth plate have same embryological origin, but their difference in reactions of these cartilage to decrease in mechanical stress hasn't been reports. This study aimed to compare and investigate differences of reactions of the articular cartilage, the growth plate and epiphyseal cancellous bone to decreasing in mechanical loads.
Materials and methods: Five weeks old rats (wistar strain, male) were used as materials. They were divided into tail-suspended group (TS) and control (CO). Furthermore, TS was divided into three groups (TS1, TS2 and TS3), CO was also divided into similar groups (CO1, CO2, CO3). TS1, TS2 and TS3 were tail-suspended for 1, 2 and 3 weeks, respectively, and CO were fed normally in same periods as TS. They were killed under euthanasia, knee joints were excised. Those structures were observed histologically and their histomorphometrical data were measured.
Results: Thickness of middle layer of articular cartilage decreased at middle and posterior portions and thickness of cartilage also decreased wholly, in CO. On the other hand, little changes of thickness of that were recognized in even TS3. Many TRAP positive cells were found at subchondral bone in TS, compared to CO. Size of chondrocytes slightly decrease in growth plate of TS, but thickness of each cell layers was same as CO.
Conclusion: It was suggested that mechanical loading might be important factor for decrease in thickness of the articular cartilage with growth, and this was defer from in the case of growth plate that related to bone growth.
(COI: No)

P1-253

Immunohistochemical localization of ectonucleotide pyrophosphatase/phosphodiesterase-1(ENPP-1) and tissue-nonspecific type alkaline phosphatase (TNALP) in bone

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Introduction: Ectonucleotide pyrophosphatase/phosphodiesterase-1 (ENPP-1) is an enzyme which synthesizes a pyrophosphate - an inhibitor of mineralization -, *e.g.*, from extracellular adenosine nucleotide. In contrast, tissue-nonspecific alkaline phosphatase (TNALP) divides a pyrophosphate into two PO4-. Both molecules are involved in bone mineralization, so that, in this study, we have examined the immunolocalization of TNALP and ENPP-1 in bone.
Materials and Methods: Male C57BL/6 mice with the age of around 8-weeks old (control group) and the mice injected with hPTH (1-34) with a regimen of 80μg/kg/day, twice/day (PTH group) were fixed, and their femurs were embedded into paraffin for histochemical detection of ENPP-1 and TNALP.
Results and Discussion: In the femoral metaphyses, TNALP-immunoreactivity was seen mainly in osteoblasts and overlying preosteoblasts, while ENPP-1 positivity was observed in osteoblasts and osteocytes embedded in the bone matrix. Unlike the control group, PTH-administered femora showed many trabeculae surrounded with a thick layer of preosteoblasts. An intense TNALP reactivity was detected in the thick layer of preosteoblasts and plump osteoblasts. Interestingly, ENPP-1 immunoreactivity was observed in some groups but not all of osteoblasts, as well as osteocytes. Thus, TNALP tends to localize preosteoblasts and osteoblasts, while ENPP-1 is seen in mature osteoblast and osteocytes.

(COI: No)

P1-254

Ultrastructural phenotypes of preosteoblasts and bone marrow stromal cells in tibial metaphyses in mice

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Purpose: Preosteoblasts are identified as osteoblastic precursors, which are localized over the mature osteoblasts and able to proliferate. Scott has verified ultrastructures of preosteoblasts by using 3H-thymidine electron microscopic autoradiography, but their ultrastructural phenotypes are still veiled. In addition, it is difficult to distinguish preosteoblasts from bone marrow stromal cells. In this study, we examined ultrastructures of preosteoblasts in the murine metaphysis.
Materials and Methods: Eight weeks-old ICR mice were perfused with a mixture of paraformaldehyde and glutaraldehyde solution, and then tibiae were extracted for additional immersion with the same fixatives. The specimens were decalcified with 5% EDTA and embedded into epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate prior to TEM observation.
Results and discussion: Bone marrow stromal cells showed flattened or spindle shapes, possessing lysosomes, lipid droplets and Golgi apparatus. In contrast, there were at least, in part, two phenotypes of preosteoblasts: one is a cell with well-developed rough endoplasmic reticulum (rER) and Golgi apparatus, which suggested their potential to synthesize extracellular matrix. The others lacked abundant rER, but included many vesicles and Golgi apparatus. Thus, our ultrastructural study suggests, at least, two distinct phenotypes of preosteoblasts in murine metaphyses.

(COI: No)

P1-255

Disrupted alveolar bone surrounding tooth germs in transgenic mice overexpressing parathyroid hormone-related peptide (PTHrP)

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Purpose: Parathyroid hormone-related peptide (PTHrP) has been reported to play a pivotal role in the development of the tooth germs, as well as bone and cartilage development. In order to verify to which tissues PTHrP predominantly affect the biological function, we have examined tooth germs and surrounding alveolar bone in mandibles of PTHrP overexpressing transgenic (Tg) mice.
Materials and Methods: Tg mice overexpressing PTHrP were generated by inserting the PTHrP cDNA downstream type I collagen promoter specific to osteoblasts. Mandibles of E18 fetuses embedded in paraffin were histochemically examined for tissue nonospecific alkaline phosphatase (ALP), tartrate-resistant acid phosphatase (TRAP), and ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1).
Results and discussion: PTHrP Tg mice showed no obvious histological abnormality of the teeth germs of molars, which revealed normally-developed enamel organs encompassed by dental follicules. But instead, there was only a few surronding alveolar bone and a huge amount of ALP-positive preosteoblastic cells throughout accompanied with a few blood vessels. ENPP1-positive mature osteoblasts and TRAP-reactive osteoclasts were shown to focally accumulate the alveolar bone surfaces. Meckel's cartilage did not seem to be enlarged. Thus, our histological findings suggest that, unlike previous reports, PTHrP would regulate cell proliferation/differentiation in alveolar bone rather than tooth germs at the fetal stage.

(COI: No)

P1-256

Ultrastructural observation on osteoblasts and osteocytes in c-fos deficient mice

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Purpose: Mature osteoblasts synthesize bone matrices, with being differentiating into osteocytes. Unlike wild-type mice, *c-fos* deficient (*c-fos*$^{-/-}$) mice lack osteoclast, and therefore, cell coupling between osteoclasts and osteoblasts does not take place. Taken together, we have attempted to verify the ultrastructural features of osteoblasts and osteocytes in the circumstance lacking cell coupling from osteoclasts.
Materials and Methods: Twelve weeks-old *wild-type* and *c-fos*$^{-/-}$ mice were perfused with an aldehyde solution, and then, femora were extracted. The femoral specimens were decalcified with 5% EDTA and embedded into epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate prior to TEM observation.
Results and Discussion: *Wild-type* mice showed mature osteoblasts with abundant rER and Golgi apparatus throughout the plump cell bodies, while *c-fos*$^{-/-}$ mice did not have such mature osteoblasts. But, instead, *c-fos*$^{-/-}$ trabecules localized bone marrow cells and fibroblast-like cells with many vesicles extending their cytoplasmic processes. Despite no mature osteoblasts, *c-fos*$^{-/-}$ specimens had osteocytes including fewer cell organelles with the nucleus becoming more prominent, and the lacunar walls showed lamina limitans, electron dense organic materials. Taken together, cell differentiation into mature osteoblasts appears to be disturbed due to a lack of osteoclasts, and the osteocytes in *c-fos*$^{-/-}$ mice seem to be previously embedded in bone matrix during their modeling period.

(COI: No)

P1-257

Generation of *tardbp* deficient zebrafish using CRISPR/Cas9 system

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Transactive response DNA binding protein-43 (*tardbp*) gene is one of responsible genes of Familial Amyotrophic Lateral Sclerosis (FALS), which is characterized by the loss of upper and lower motor neurons. In this study, we knocked out zebrafish *tardbp* and its paralogue *tardbp*-like (*tardbpl*) genes using the CRISPR/ Cas9 system to analyze the mechanisms of neural degeneration in ALS patients and relationship between the neurogenesis of motor neurons and angiogenesis. Although *tardbpl* gene lacks the glycine-rich domain where many ALS associated mutations are reported, it is upregulated to compensate *tardbp* function in its mutant. So we designed and synthesized sgRNAs for the targeted sequence of both *tardbp* and *tardbpl* genes. Genome DNAs were extracted from the sgRNA-injected embryos at 1 day post fertilization (dpf) and then analyzed the activity for genome modification by Heteroduplex Mobility Assay (HMA). Furthermore, we analyzed the vascular morphogenesis in *tardbp* and *tardbpl* mutant using *Tg(fli1a:EGFP)*^{y1} embryos, in which the endothelial cells specifically expressed the EGFP. As a result, we succeeded to confirm the activity of each sgRNA for targeted genome editing by HMA analysis and observe aberration of intersegmental vessel in sgRNAs and Cas9 mRNA injected embryos at 2dpf. In future, we will generate homozygotic *tardbp* and/or *tardbpl* mutant zebrafish and elucidate mechanism of neural degeneration in ALS and the involvement of neurogenesis in angiogenesis.

(COI: No)

P1-258

Cyclin B3 is involved in leg regeneration of the cricket *Gryllus bimaculatus*

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Several animals such as planarian, cricket and newt have remarkable regenerative capacity to restore the lost part of limb completely although regenerative capacity of higher vertebrate including human is limited. Almost all genes are conserved in the genomes between both regenerative animals and non-regenerative animals, suggesting that regenerative capacity of human could be regained by modifying gene(s) function. The cricket *Gryllus bimaculatus* is an emerging model animal for regeneration biology. When we amputate leg of cricket nymph, cricket regenerates the lost part of leg through several molts. Our previous studies showed that tumor suppressor Hippo pathway suppresses cell proliferation during regeneration. In this study, we focus on the function of cyclin B3, which is a target of Hippo pathway, during regeneration. We cloned partial fragments of *Gryllus* homologue of *cyclin B3* (*cycB3*) and performed RNAi. The amount of *cycB3* mRNA was decreased by RNAi, but amount of *cycB* mRNA was not decreased as revealed by quantitative PCR. In *cycB3*(RNAi) cricket, lost part of leg was regenerated but the size of regenerate was smaller than that of control. These results suggest that cyclin B3 may promote cell proliferation during regeneration to restore the lost part. We cloned partial fragments of *Gryllus* homologue of *cycB*. We will discuss cooperative functions of *cycB3* and *cycB* during regeneration.

(COI: No)

P1-259

Chronological changes in prosaposin receptors immunoreactivity in rat brain after birth

Yorozuya, Aika[1]; Nabeka, Hiroaki[2]; Shimokawa, Tetsuya[2]; Khan, Md S[2]; Li, Xuan[2]; Doihara, Takuya[2]; Yamamiya, Kimiko[2]; Hamada, Fumihiko[3]; Kobayashi, Naoto[4]; Matsuda, Seiji[2] (*[1]Ehime Univ Sch Med, Toon, Japan; [2]Anat Embryol, Ehime Univ Grad Med, Toon, Japan; [3]Anat, Oita Univ F Med, Yufu, Japan; [4]Education C, Ehime Univ Grad Med, Toon, Japan*)

Prosaposin (PSAP) is the precursor of saposins A-D. Many reports suggest that PSAP is a neurotrophic factor in vivo and in vitro that induces differentiation and prevents death in a variety of neuronal cells. We previously reported the chronological changes in PSAP immunoreactivity and in the mRNA expression of PSAP in developing rat brain using in situ hybridization. Abundant PSAP expression in the perinatal stages indicates a potential role for prosaposin in early rat brain development (Xue et al. 2011). Recently, the G protein-coupled receptors GPR37 and GPR37L1 were recognized as PSAP receptors. In the present study, we examined changes in immunoreactivity against the PSAP receptors GPR37 and GPR37L1 in rats. In rat brain at 1, 2, or 4 weeks after birth, many neurons in the cerebral cortex showed intense or weak immunoreactivity against GPR37 and GPR37L1. In particular, in rat cerebral cortex at 1 week after birth, neurons in the lower layers showed intense immunoreactivity. Also, in the hippocampus and dentate gyrus, many neurons with receptor immunoreactivity were observed.

(COI: No)

P1-260

Deletion of ATF6α enhances Kainate-induced neuronal death in mice

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High level of glutamate results in neuronal degeneration/death in various pathological conditions including epilepsy and stroke. We previously reported that ORP150, a molecular chaperone in the endoplasmic reticulum (ER), protected hippocampal neurons against glutamate-induced neuronal death (Kitao et al., 2001). However, the role of ATF6 α, a transcriptional factor important for the chaperone expression, was not clear yet in such situations. We, therefore, analyzed the activating status and the role of ATF6 α in a mouse model of glutamate-induced neuronal death. When kainate (KA), a strong agonist of glutamate receptor, was injected into the CA3 region of the hippocampus in wild-type (WT) mice, enhanced expression levels of ORP150 and GRP78, both are downstream gene products of Atf6 α, were observed, suggesting activation of ATF6 α in this model. We then estimated the level of neuronal damage in both WT and Atf6 α-/- mice. Higher levels of neural degeneration and neuronal death were observed in Atf6 α-/- mice, while no significant difference were observed in glial cell activation between two genotypes. Further analysis revealed that expression level of c-fos, a marker of neuronal activity, was higher in Atf6 α-/- mice after KA injection. Injection of thapsigargin, an inhibitor of calcium uptake into the ER, also caused higher level of neuronal death in Atf6 α-/- mice. These results suggest that ATF6 α plays important roles for neuronal survival after KA injection through the regulation of calcium response and neuronal activity.

(COI: No)

P1-261

Changes of serotonin-positive neuron in rat medulla oblongata during hypoxia

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Hypoxia activates the neurons in ventral respiratory group (VRG) and dorsal respiratory group (DRG) in medulla oblongata to increase tidal volume and respiratory frequency. It is reported that concentration of serotonin (5-HT) rise in the VRG and DRG during hypoxia. Thus we immunohistochemically examined changes of serotonergic neurons in medulla of rats exposed to hypoxia (10% O2) for 1, 2, 4 and 6 hr. Using antibodies against Fos, 5-HT, tryptophan hydroxylase 2 (TPH2) and Ser-19 phosphorylated TPH2 (pTPH2). In the rat exposed to hypoxia, Fos-labeled neurons were observed in the paragigantocellular reticular nucleus, lateral part (PGRNl) in the VRG, and nucleus of the solitary tract, gelatinous part (NTSge) and medial part (NTSm) in the DRG. In PGRNl, 5-HT-immunoreactivity in the nerve fibers were significantly increased in rats exposed to hypoxia for 2, 4 and 6 hr in rostral part, and 1, 2, 4 and 6 hr in caudal part compared with control ($p<0.05$). In the NTSge and NTSm, 5-HT-immunoreactivity were increased in 2 hr ($p<0.05$). In these areas, 5-HT-immunoreactive nerve fibers were observed close to the Fos-immunoreactive cells by double immunofluorescence. On the other hand, nerve cell bodies immunoreactive for TPH2 and pTPH2 were distributed in nucleus raphe magnus, nuculeus raphe obscurus, nucleus raphe pallidus and gigantocellular reticular nucleus pars α. The number of the immunoreactive neuron in these nuclei did not change by hypoxic exposure. In conclusion, it is suggested 5-HT is increased in VRG and DRG during hypoxia to modulate respiratory drive.

(COI: No)

P1-262

A gap between adjacent surfaces deteriorates depth perception based on binocular correlation computation

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The visual system computes depth from binocular disparity. The initial encoding of disparity is achieved by computing cross-correlation between left-eye and right-eye images. When luminance contrast of either image is reversed (binocularly anti-correlated), neurons signaling the cross-correlation show inverted disparity tuning. It remains elusive whether the correlation-based signals are exploited by the brain to produce depth perception. We previously showed that anti-correlated stereograms (aRDSs) evoke reversed depth, suggesting that the brain does use the signals (Tanabe et al., 2008). However, Hibbard et al. (2014) found no reversed depth for stimuli that had a gap between a patch and its surround but were otherwise similar to ours. Here we examined effects of a gap between the two surfaces on reversed depth. Subjects were shown a concentric-bipartite RDS and reported whether the center patch was nearer or farther than the annular surround. The patch was either a contrast-matched RDS (cRDS) or an aRDS with crossed or uncrossed disparities, while the surround was always a cRDS at 0 disparity. Most subjects (8 out of 12) perceived reversed depth for aRDS patches with a gap of <0.175 deg. Reversed depth diminished as the gap became wider, and disappeared when a gap was 0.7 deg wide. A small gap thus profoundly affected reversed depth, suggesting that correlation-based depth signals are integrated over a spatially limited range of visual field.

(COI: No)

P1-263

Gradual loss of visual ability in the animal model of retinitis pigmentosa

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The Royal College of Surgeons (RCS) rat is an animal model of retinitis pigmentosa, losing the visual ability gradually over time, and has been used for the various recovery studies. However, the time course of the visual loss has not been examined quantitatively in a behavioral test because visual degradation is possibly progressive before RCS rats complete the learning of the behavioral task. Moreover, it remains unknown how the visual responses of neurons in the geniculocortical pathway diminish with time. To answer these questions, we established a new method of task-training enabling one-week learning of the two-alternative forced-choice visual grating detection task. Rats were trained at 4 weeks of age, and measured the contrast threshold from 6 to 11 weeks of age every day. We found that the visual ability of pattern vision was diminished from 7 to 8 weeks of age, and thereafter, fell to an unmeasurable level. To examine the neuronal basis of the grating detectability degradation, we conducted the extracellular recordings from the dorsolateral geniculate nucleus (dLGN), the primary visual cortex (V1), and the superior colliculus (SC) of awake RCS rats at various ages, finding that the visual responses to grating stimulus in these regions were decreased in a similar time course to the behavioral performance. Now, we are conducting the additional measurement of behavioral and neuronal visual sensitivity to brightness vision using a high luminous flash stimulus.

(COI: No)

P1-264

Schizophrenia-relevant symptoms were displayed in *Zinc Finger Protein 521 (ZFP521)* knockout mice

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Zinc finger protein 521 (ZFP521) in the mice, also known as ZNF521 in humans, is a nuclear protein. ZFP521 regulates the differentiation of several kind of stem cells in a wide range of tissue, such as osteoblast formation and adipose commitment and differentiation. In the field of neurobiology, it is reported ZFP521 is an essential factor for transition of epiblast stem cells into neural progenitors *in vitro*. However, the role of *ZFP521* in the brain *in vivo* still remains elusive. To elucidate the role of ZFP521 in the mouse brain, we generated *ZFP521* knockout (*ZFP521*$^{-/-}$) mice and analyzed them in detail.

Although *ZFP521*$^{-/-}$ mice were smaller than *ZFP521*$^{+/+}$ and *ZFP521*$^{+/-}$ littermates, they had no apparent defect in the body. They displayed abnormal behavior, such as hyper-locomotion, lower anxiety, impaired learning and deficits in prepulse inhibition, which correspond to the symptoms of schizophrenia. The border of the granular cell layer of the dentate gyrus in the hippocampus of the mice was indistinct and granular neurons were reduced in number. Furthermore, Sox1-positive neural stem cells in the dentate gyrus and cerebellum were significantly reduced in number. Taken together, these findings indicate that ZFP521 affects the formation of the neuronal cell layers of the dentate gyrus in the hippocampus, and thus *ZFP521*$^{-/-}$ mice displayed schizophrenia-relevant symptoms.

(COI: No)

P1-265

Functional characterization of FTSJ1, a X-linked mental retardation-related gene

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Genetic mutations in X chromosome-linked genes have been associated with mental retardation (XLMR). Recently, linkage analyses performed in Belgian, Chinese and Japanese families have identified Ftsj1 gene as a novel candidate gene. Ftsj1 shares homology with a bacterial 23S rRNA methyltransferase FTSJ. However, the molecular function of Ftsj1 and its pathological relevance in mental retardation have remained unknown. Using Ftsj1 knockout mice, we demonstrate that Ftsj1 methylates cytosolic transfer RNAs (tRNAs) at position 32 and 34. While the FTSJ1 KO mouse developed normally, we observed a decreased protein synthesis level in hippocampus of FTSJ1 KO mice using puromycin-mediated in vivo pulse-labeling technique. Especially, there was a marked decreased of synaptic proteins including glutamate receptors and signaling molecules. The decreased protein synthesis level resulted in the electrophysiological and morphological abnormalities in hippocampal neurons of FTSJ1 KO mice. There results suggest that the accumulation of hypomodified tRNAs disturbs neuronal protein synthesis, which ultimately contributes to the development of mental retardation in Ftsj1-deficient mice and human.

(COI: No)

P1-266

Spatiotemporal recalibration of inferred motion in monkeys

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Even when a moving object is temporally occluded behind a stationary object, we can precisely predict when and where it reappears. Previous studies suggest that both the parietal cortex and the cerebellum are involved in the inference of visual motion. As a step toward understanding the neural mechanism, we have developed a behavioral paradigm that requires spatial and temporal recalibration of inferred motion. Experiments were conducted on two Japanese monkeys. A target spot moved obliquely at 20°/s. After 500 ms, it was occluded behind a stationary rectangle that was visible throughout the trial. Monkeys were trained to make a predictive saccade to the target that reappeared on the other side of the rectangle. In the spatial adaptation paradigm, the location of target reappearance was displaced by 5° horizontally. In the temporal adaptation paradigm, the target reappearance was delayed or preceded by 200 ms. These trials were presented in separate blocks. After 600 spatial adaptation trials, saccade endpoints were shifted by 3.7 ± 0.5° (SD, n = 8), while saccade timing remained unchanged. Likewise, after 600 temporal adaptation trials, saccade timing altered by 107 ± 17 ms (n = 8), while saccades remained accurate. Although adaptation of predictive saccades did not transfer to visually-guided saccades, adaptation of visually-guided saccades altered the metrics of predictive saccades. These results suggest that the spatial and temporal aspects of inferred motion might be subject to separate recalibration mechanisms, which appear to be different from the saccade adaptation mechanism in the medial cerebellum.

(COI: No)

P1-267

The secondary auditory cortex receives topological projections from the ventral division of the medial geniculate body in mice

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It is generally known that the belt region including the secondary auditory cortex (AII) receives thalamic inputs from the dorsal division (MGd) of the medial geniculate body (MGB) that is not structured tonotopically. Recently, however, a robust tonotopic structure was revealed in AII in mice. Here, we verified the possibility that the mouse AII receives topological projections directly from the ventral division of MGB (MGv) which is structured tonotopically. We identified the precise location of AII in C57BL/6 mice using flavoprotein fluorescence imaging. When 5-60 kHz tones were presented, a clear tonotopic gradient traveled ventrally. Next, we injected Alexa Fluor 488- or 555-conjugated CTB, a retrograde tracer, into a 5 or 35 kHz area in AII to investigate the location of neurons projecting from MGB to AII. Three days after injection, we performed cardiac perfusion and prepared coronal sections. Immunostaining of non-phosphorylated neurofilament (NNF) in adjacent slices was used to parcellate subdivisions of MGB. We obtained three positive results as follows. First, majority of neurons projecting to AII were located inside MGv. Second, neurons projecting to MGv were localized in the caudal part of the MGv. Finally, neurons projecting to the 5 kHz or 35 kHz area were different in location from ventrorostral to dorsocaudal. These results strongly suggest that the caudal part of MGv has a distinct tonotopic gradient, and the tonotopic gradient of AII reflects topological projections originating in the caudal part of MGv.

(COI: No)

P1-268

Effects of D/L-Valine on tongue movement in the isolated brain stem-spinal cord intact tongue preparation

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The tongue is composed of sensory system as a taste, and motor system such as mastication, swallowing and vocalization. Sense of taste on the surface of the tongue and sent to the brain can feel only sweetness, sourness, saltiness, bitterness and umami. In the rat, at first the gustatory nerve connects to 1) the solitary tract nucleus, toward reticular formation; 2) the solitary tract nucleus, toward parabrachial nucleus and reaches taste area in cortex. We designed the preparation remained taste circuit keeping sensory-motor connection, so we produced isolated brain stem-spinal cord intact tongue preparation including solitary tract nucleus, parabrachial nucleus, facial nucleus. Moreover, we examined the effects of sweet amino acid D-valine and bitter amino acid L-valine on tongue muscle activity as a tongue movement. The tongue movement was recorded by bipolar-tungsten electrode inserted to tongue muscle. Application of D-valine to tongue increased tongue movement after 5-10minutes from application, but L-valine inhibited tongue movement or showed long delayed effect in postnatal 0-2-day-old rat. We also examined developmental changes of D-/L-valine effects. In the embryonic day 16 (E16), the tongue movement was irregular and D-/L-valine effects were invisible, but in the embryonic day 18 (E18), the tongue movement detected clearly and the effects of that were seen. These results suggested that we succeeded the useful preparation for analysis of taste circuit, and we showed difference influence of between bitter and sweet sense.

(COI: No)

P1-269

Developmental changes in synaptic plasticity of the hippocampal CA1 neurons by contextual memory

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Neural mechanisms of brain keep changing during the course of a lifetime. Although our previous study showed a developmental relationship between contextual learning and dorsal hippocampal ACh levels (Takase et al., Sci Rep, 2014), it has not revealed the developmental changes of post-synaptic currents by contextual learning. In this study, we compared miniature excitatory and inhibitory post-synaptic currents (mEPSC and mIPSC) between contextual learning trained and untrained rats in 3-weeks, 4-weeks, 6-weeks and 8-weeks-old. As a learning model, we employed inhibitory avoidance (IA) task, and acute brain slices were prepared for patch clamp analysis. For untrained rats, 6-weeks and 8-weeks-old revealed higher mEPSC frequency than 3-weeks-old. Whereas, after employing IA task, 4-weeks-old trained rats showed higher mEPSC amplitude than that of untrained rats. In addition, IA trained rats showed higher mIPSC amplitude than untrained rats in 4-weeks, 6-weeks and 8-weeks-old. These results suggest that hippocampal CA1 synapses change with development of brain and contextual learning has a strong effect on CA1 synapses in 4-weeks, 6-weeks and 8-weeks-old rats. Thus, we conclude that the strengthening of hippocampal CA1 synapses by contextual memory may differ in developmental stages.

(COI: No)

P1-270

Changes in hippocampal CA1 neuronal activity and sympathetic nerve activity during fear conditioning in rats

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Hippocampus has been implicated in the emotional responses of sympathetic nerve acidity to fear, however there has been lack of direct evidence on the changes hippocampal neuronal and sympathetic nerve activity and on functional relationships between those activity during development of fear. The aim of the present study was to measure relationship between hippocampal neuronal activity and sympathetic nerve activity during fear conditioning. Wistar male rats were instrumented chronically with multiple electrodes for hippocampal CA1 neuronal activity and bipolar electrode for renal (RSNA) and lumbar sympathetic nerve activity (LSNA) and electroencephalogram. We gave each rat 5-sec tones co-terminating with a 1-sec, 5-mA foot shock twice a day that was carried out over 3 days. During the fear conditioning over 3 days, changes in hippocampal CA1 neuronal activity, RSNA, LSNA were measured simultaneously during quiet awake state in the rat's home cage. Heart rate decreased progressively due to the fear conditioning over 3 days. Hippocampal CA1 neuronal activity and RSNA increased due to fear conditioning, while LSNA decreased during fear conditioning. These data suggest that there is a neuronal positive coupling between hippocampal neural activity and RSNA during fear conditioning in rats.

(COI: No)

P1-271

Slow depolarization induced by dopamine in spinal motor neurons

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Dopamine plays important roles as a slow neurotransmitter in the nervous system. Dopamine was reported to have an excitatory effect on motor coordination (Whelan et al., 2000). The cellular mechanisms of dopamine action on the motor neurons and the origin of dopamine are not clarified yet. We studied the effect of exogenous dopamine on the membrane properties of the motor neurons in the organotypic slice culture of rat spinal cord prepared from P0 rat. Bath application of dopamine (10 μM) induced slowly developing depolarization in a motor neuron under current-clamp (peak amplitude of the depolarization, 11.2 ± 3.4 mV, n=4) whether tetrodotoxin (1 μM) was present in the bath solution or not, indicating that a postsynaptic mechanism is involved in the dopamine- induced depolarization. Then the effect of dopamine on the membrane conductance of a motor neuron under voltage clamp was studied with a potassium gluconate-based solution in the patch pipette. In the presence of tetrodotoxin (1 μM), the membrane currents were measured at different holding voltages (from -90 to +40 mV) under voltage-clamp before application of dopamine and after the inward currents reached a plateau level. The relationship between the change of the membrane currents and the holding potentials revealed that the change of the membrane currents was due to the reduction of the outward currents with a reversal potential, close to an equilibrium potential for potassium, -86 mV, indicating reduction of a potassium conductance.

(COI: No)

P1-272

Involvement of 5-HT_6 receptor in local feedback inhibition of the dorsal raphe serotonergic neurons

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In the dorsal raphe nucleus (DRN), many GABAergic neurons project to serotonergic (5-HT) neurons and regulate their activity. However, the mechanisms how such local inhibition is maintained remain unclear. In this study, we examined the roles of 5-HT receptors in the local GABAergic inhibitory circuits regulating 5-HT neuronal activity. In the organotypic raphe slice cultures, a $GABA_A$ receptor antagonist, bicuculline, increased 5-HT release. Similarly, an atypical antipsychotic, olanzapine, which potently antagonizes some 5-HT receptors, increased 5-HT release, and this effect was occluded in the presence of bicuculline. Among 5-HT receptors to which olanzapine has higher affinity, a 5-HT_6 receptor antagonist, SB399885, but not 5-HT_{2A} and 5-HT_{2C} receptor antagonists, significantly increased 5-HT release. Like olanzapine, SB399885 did not show further increase in 5-HT release in the presence of bicuculline, suggesting the involvement of GABA inhibitory inputs. Moreover, in acute raphe slice, both olanzapine and SB399885 significantly decreased spontaneous firing of the DRN Gad2-positive neurons, in which 5-HT_6 receptor mRNA was expressed. These results suggest that 5-HT_6 receptor plays an important role for maintaining activity of DRN GABAergic neurons as a feedback regulation of DRN 5-HT neurons.

(COI: No)

P1-273

Synchronized high frequency oscillation and the theta oscillation between the hippocampus and the amygdala after fear conditioning correlates freezing behavior

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Memory consolidation process occurs during rest stage including slow-wave sleep by sharp wave-ripple complex (SWRs) that observed in CA1 region of the hippocampus (HPC). The SWRs interaction of BLA and HPC to fear conditioning was unknown. To find out about this issue, we investigated the relationship of the freezing behavior reflecting fear memory and the synchronization of the high frequency oscillations from HPC and BLA during rest time. After recovery from the electrode implantation, rats were placed into the test box for foot shock. After the foot shock, rats were returned to their home cage, and local field potentials of HPC and BLA were recorded for 40-50 minutes. After 1 hour from foot shock, rats were placed into the test box again and record the freezing behavior. The synchronized high frequency oscillations (100-250 Hz) indicating SWRs between HPC and BLA were observed during the rest time at the home cage after foot shock. The synchronized theta oscillation with the synchronous events was observed in the BLA. This synchronized theta oscillation power showed a negative correlation to the freezing behavior. These results suggest that the HPC ripple affects the theta oscillation of the BLA and is involved in fear memory consolidation.

(COI: No)

P1-274

Multiple free-radical scavenging activity of alpha lipoic acid derivatives: An ESR study

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Objectives: α-lipoic acids are illustrated to have antioxidant activity in various ischemia/reperfusion models, but few studies reveal against which free radical species they have radical scavenging activity. In this study, using electron spin resonance spectrometry (ESR), we directly evaluated spectra of free-radical scavenging activity of α-lipoic acid derivatives and estimated IC_{50}.
Methods: We evaluated the following recently synthesized water-soluble α-lipoic acid derivatives: dihydrolipoate (DHL)-taurine-Zn complex, DHL-penicillamine-Zn complex, DHL-glutamate-Zn complex, DHL-norleucine-Zn complex, DHL-anthranilate-Zn complex, DHL-histidine-Zn complex, and DHL-Zn complex. Direct free radical scavenging activity was evaluated for the following free radical species; hydroxyl radical, superoxide anion, *t*-butyl peroxyl radical, ascorbyl free radical (AFR), 1, 1-diphenyl-2-picryl hydrazyl radical, and nitric oxide. *g*-CYPMPO, DMPO and c-PTIO were used as spin traps. Peroxidation in brain homogenate was evalutated by TBARS assay.
Results and Conclusion: All α-lipoic acid derivatives examined indicated concentration-dependent radical scavenging activity against all radicals examined except AFR. Although IC_{50} varied among radical species, antioxidant activity of α-lipoic acid derivatives is, at least, partially attributable to their direct free radical scavenging activity against multiple free radical species.

(COI: No)

P1-275

Changes in the neurons of the spinal cord in a chick model of spina bifida aperta

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Spina bifida aperta (SBA), a neural tube defect that occurs during embryonic development, is one of the most common human congenital defects of the central nervous system. It involves protrusion of the spinal cord and/or meninges through a defect in the vertebral arches and skin. Depending on the position of the lesion, SBA causes postnatal physical disabilities, including paralysis of the legs, a lack of bowel and bladder control, and hip, knee, and foot abnormalities after birth. Although the etiology of SBA remains unknown, the pathogenic mechanism is generally thought to be a disorder of neurulation, with a failure of neural plate closure. The leg dysfunction that occurs in this model was reported to be the result of a decrease in the number of interneurons at the spinal segments, which modulate the motor neurons that innervate dysfunctional muscles (Mominoki et al., 2006). These findings prompted us to further investigate abnormalities in the spinal motor neurons. We created SBA chicks by incising the roof plate of the neural tube in the embryo and studied the pathological changes in the spinal cord. Histological analyses revealed large spinal neurons, most likely motor neurons, at the level of the lesion in the SBA chicks with an irregular configuration compared to the normal control chicks. The number of large neurons did not differ between the SBA and control chicks, but the large neurons were densely packed.

(COI: No)

P1-276

Prosaposin and its receptors in the facial nucleus after facial nerve transection

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Prosaposin is the precursor protein of four small lysosomal glycoproteins known as saposins (saposin A-D). In addition to its role as a precursor protein, prosaposin acts as a neurotrophic factor. Both saposins and prosaposin are widely expressed in various tissues, although the brain, skeletal muscle, and heart cells predominantly contain unprocessed prosaposin rather than saposins. Prosaposin and prosapotide, a peptide containing the neurotrophic activity domain of prosaposin, promote neurite outgrowth, elevate choline acetyltransferase activity in neuroblastoma cells, and prevent programmed cell death in cultured neurons. We previously detected increases in prosaposin immunoreactivity and the expression of prosaposin mRNA in the rat facial nerve nucleus following facial nerve transection. Prosaposin mRNA expression increased not only in facial motoneurons, but also in microglia during facial nerve regeneration. In the present study, we examined the change in immunoreactivity of the prosaposin receptors GPR37 and GPR37L1 in the rat facial nucleus following facial nerve transection. In the facial nucleus on the transected side, many small cells, most likely glial cells, with strong GRP37L1 immunoreactivity were observed.

(COI: No)

P1-277

The new spontaneous mutant mouse allele for *dystonia musculorum*

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The spontaneous mutant mouse showing twisting body and limbs was found in the colony of Riken BRC (Riken *dt* mouse). Linkage mapping analysis suggested the chromosome 1 is mutated and this mouse is the new candidate allele for *dystonia musculorum* (*dt*) with abnormal *dystonin* (*Dst*) gene. To confirm this hypothesis, Riken *dt* mouse was mated with the newly created *Dst* gene trap mouse (Dst^{Gt}), in which actin-binding domain-containing isoforms are disrupted. Dst^{Gt} homozygotes show *dt* phenotype as Riken *dt* mouse but heterozygote is normal. Several littermates obtained from the heterozygous parents of Riken *dt* and Dst^{Gt} mice showed twisting body and limbs. These littermates also show the abnormal neurofilament staining on the sections in the nervous system. These results strongly suggested that Riken *dt* mouse is new *Dst* allele.

(COI: No)

P1-278

Netrin-5 is highly expressed in neurogenic regions of the adult brain

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Mammalian netrin family proteins, netrin-1, -3, and -4, are involved in targeting of axons, neuronal migration, and angiogenesis and act as repulsive and attractive guidance molecules. Netrin-5, a new member of the netrin family, has homology to the C345C domain of netrin-1. Unlike other netrin proteins, murine netrin-5 consists of two EGF motifs of laminin V domain (LE) and the C345C domain, but lacks the N-terminal laminin VI domain and one of the three LE motifs. Interestingly, netrin-5 is strongly expressed in the olfactory bulb, rostral migrate stream (RMS), the subventricular zone (SVZ), and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus, where neurogenesis occurs in the adult brain. Doublecortin (DCX)-positive neuroblasts co-express netrin-5 in both the SVZ and RMS, whereas GFAP-positive astrocytes do not. In the SGZ, DCX-positive neuroblasts co-express netrin-5, indicating that netrin-5 expression occurs in at least type 2b to type 3 cells. In type 1 cells, GFAP does not show co-localization with netrin-5 in the SGZ. Overall, these expression patterns of netrin-5 support the hypothesis that this molecule may play a fundamental role in adult neurogenesis. In addition, we also show developmental expression pattern of netrin-5.

(COI: No)

P1-279

Response of human mesenchymal stem/progenitor cells (hMSCs) on ischemic hippocampal homogenate

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Cerebrovascular disease is a devastating disease and places third position of the death in Japan. Observations made on animal models suggest that a potential therapy for disorders of the disease is the administration of adult hMSCs. However, it is still unclear how the hMSCs response in the implanted brain. To solve the issue, we conducted hMSCs culture incubating with ischemic brain homogenate (ibCM) or non-ischemic brain homogenate (bCM). Global ischemia was induced by 15 min transient common carotid artery occlusion. One day after, the hippocampus were removed and homogenated with α-MEM based medium (CM). The supernatant was diluted to 0.5mg/ml with 1% FBS containing CM, and applied to sub-confluent cultured hMSCs. The cells were collected and analyzed the gene and protein expressions by omix analysis at 1day. Furthermore, the total and dead cell numbers were counted. MSCs were immunostained 24-factors which were selected from the gene expressions. No differences were observed the cell numbers between ibCM and bCM exposure. ibCM hMSCs increased 98 genes and decreased 78 genes more than 2-fold. Immunocytostaining was confirmed an increment of CXCL1, CCL2, IL-6, Mn-SOD, HuD, thioredoxin1, amyloid β in ibCM hMSCs. Semi-quantification with chemokine protein array also determined an increase of them. These results suggest that transplanted hMSCs could communicate with host tissues/cells mediated by chemokines.

(COI: No)

P1-280

Oct-3/4 induces expression a DNA-repair enzyme O6-methylguanine-DNA methyltransferase (MGMT) through its epigenetic effects in glioblastomas

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Alkylating agents, such as temozolomide, are the most effective agents for the treatment of malignant gliomas. A cellular DNA-repair enzyme, MGMT reverses alkylation at the O6 position of guanine, thereby the expression level of MGMT is closely related to the sensitivity of brain tumors for alkylating agents. MGMT expression is controlled by a methylation/demethylation of the cytosine phosphate guanosine (CpG) islands in the promoter region of MGMT gene. Oct-3/4, a self-renewal regulator in stem cells, has been known to express on various kinds of solid tumors including glioblastoma, and has been involve in tumor progression, malignancy in glioblastomas. However, little is known regarding the MGMT expression in glioblastoma. We investigated whether Oct-3/4 involves in the sensitivity of temozolomide through the expression of MGMT in this study. Oct-3/4 overexpression resulted in decreased susceptibility to temozolomide thata accompanied upregulated expression of MGMT mRNA. As analyzed by genomic sequencing of bisulfate-modified DNA, Oct-3/4-expressing cells showed enhanced demethylation of CpG islands. In glioblastoma patients, Oct-3/4 expression were well correlate with the expression of MGMT mRNA and CpG-demethylation status of its promoter region. These results suggest that Oct-3/4 promotes the resistance against temozolomide of glioblastoma cells by upregulating MGMT expression through the epigenetic change of MGMT promoter region.

(COI: No)

P1-281

Analyses of the recruitment mechanism of TGFβ activity in the invasive glioma tissue

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Insidious invasions of glioma cells from primary tumor lesion toward surrounding normal brain parenchyma cause untreatable situations at the recurrence after surgical resection. We have established the insidious invasion model in KSN nude mice by xenografting C6 rat glioma cells into the brain, and observed the invasion accompanied by CD105/Endoglin (TypeIII TGF β receptor) positive while CD309/VEGFR2 negative blood vessels, implying dominant contribution of TGF β 1 rather than VEGF in insidious glioma invasions. The most promising candidate for the source of TGF β might be the primary tumor mass. The mRNA levels of TGF β 1 were markedly higher in the primary tumor tissues than in non-tumor counter hemispheres. Primary tumor lesions are constituted not only but with significant amount of tumor associated macrophage-like cells (TAM). In comparison of the mRNA levels of primary cultured TAM and C6 cells, former was the dominant. However, since conversion from the latent form to the active form by protein processing is essential for TGF β to act, measurement of TGF β activity is indispensable to know the molecular mechanism of recruitment of the TGF β in glioma invasions. We are on the way to determine the dynamics of the activity through assessments of culture media and ECMs where latent form of TGF β deposits. We would like to discuss about therapeutic possibility of the source of TGF β and the molecular mechanisms of the recruitment of the activities.

(COI: No)

P1-282

Quantitative analysis of axonal elongation and dynamics of neuronal mitochondria by using confocal time-lapse imaging

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Neuronal migration and axonal maturation occur during the construction of neural network. The process of axonal elongation needs proper transport and localization of proteins, lipids, and organelles. It is known that mitochondria produce ATP and localize at growth cones, and inhibiting mitochondrial transport and localization causes abnormal formation of axons. However spatial and temporal relation between mitochondrial dynamics and morphological development of axon is poorly understood. In order to study this relationship, we performed confocal time-lapse imaging using dissociated hippocampal neurons expressing mitochondrial marker (mitochondrial outer membrane protein Omp25C tagged with EGFP; OMP-EGFP) and DsRed2. By analyzing 12-hour-time-lapse imaging with 5 min intervals quantitatively, we could investigate the spatiotemporal relationship between axonal elongation and mitochondrial dynamics. The distribution of neuronal mitochondria changed following axonal elongation but mitochondria showed a tendency to position in the proximity of growth cones. This result suggests the presence of a mechanism that regulates positioning of mitochondria close to the advancing growth cones, which may consume more energy than other parts of the growing axons.

(COI: No)

P1-283

Molecular mechanism of phagosome formation by Rac1switching control both in space and time

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Rac1, a G-protein molecular switch, controls actin organization and mediates actin-based cell motilities such as pseudopod extension, ruffling and phagocytosis. However, significance of Rac1 switching between ON and OFF in Fc γ R-mediated phagocytosis is unknown. Therefore, we elucidated the roles of Rac1 activation/deactivation in the process of phagosome formation using quantitative assay, live-cell imaging, immunofluorescence and scanning electron microscopy of RAW264 macrophages expressing YFP-fused Rac1 mutants. Also, optogenetics of photoactivatable Rac1 (mCherry-LOV-Rac1-Q61L) was applied to reversible control of the molecular switch. As well as dominant negative Rac1-T17N, constitutively active Rac1-Q61L inhibited phagocytosis of IgG-opsonized erythrocytes (IgG-Es). Live-cell imaging and scanning EM of Rac1-Q61L-expressing cells demonstrated that ruffle-like pseudopodia were formed around IgG-Es bound on the cell surface, however, formation of phagocytic cups grasping IgG-Es was restrained. Immunofluorescence microscopy showed that phosphorylation of myosin light chain was reduced by activated Rac1, indicating activated Rac1 inhibits myosin II. Furthermore, optogenetic analysis revealed that Rac1 activation extends pseudopodia around IgG-Es, and its deactivation circularly constricts the pseudopodia to grasp IgG-Es. These findings suggested that Rac1 activation is crucial for pseudopod extension through actin polymerization, but subsequent deactivation is also required for contractile activities by myosin II to shape phagocytic cups.

(COI: No)

P1-284

Sequential recruitments of Rab35, Rab8 and Rab10 during macropinosome formation

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Macropinocytosis is a fluid-phase endocytic process that forms relatively large vacuoles called macropinosomes. Its processes consist of membrane ruffling, circular ruffle (macropinocytic cup) formation and then separation from the plasma membrane as macropinosomes by cup closure. In this study, we elucidate whether several Rab proteins (Rab1, Rab8, Rab10, Rab12, Rab13, Rab15, Rab35) are involved in macropinocytosis by live cell imaging. RAW264 macrophage cells were transiently expressed GFP-fused Rab proteins and observed using by a confocal microscope. As a result, Rab8, Rab10 and Rab35 were recruited to local sites of macropinosome formation. Rab35 localized in ruffle membranes and dissociated from the membrane during macropinocytic cup formation, whereas Rab8 and Rab10 were transiently recruited to the membrane of macropinocytic cup and then disappeared. We compared the timing of recruitment of Rab8 and Rab10 by co-expression experiments. Rab8 was slightly earlier recruited to the cup than Rab10. Moreover, dominant negative mutant of Rab10 or Rab35 inhibited macropinocytosis. These results suggested that sequential recruitments of Rab35, Rab8 and Rab10 play crucial role in macropinosome formation.

(COI: No)

P1-285

Time-lapse imaging of endosome acidification using pH-responsive fluorescent organosilica nanoparticles

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Cellular phagocytosis plays an important role in life activities such as uptake and degradation of pathogens. After phagocytosis the inside of endosome is acidified by proton pumps to degrade the contents. The detailed analysis of the endosome acidification is important in understanding the life phenomenon.

To understand the process from phagocytosis to acidification, we performed time-lapse imaging of the endosome acidification of the macrophage.

We prepared pH-responsive fluorescence organosilica nanoparticles. A pH-responsive fluorescence dye, AcidiFluor™ ORANGE was attached to the surface of thiol-organosilica nanoparticles containing FITC.

We added the nanoparticles and observed the phagocytosis and endosome acidification of the RAW264.7, a macrophage cell line, with time-lapse microscope. We measured two kinds of fluorescence intensities, inside of nanoparticle and AcidiFluor™ ORANGE on the surface of particles, in single cell at the same time. The ratio analysis was performed using these two kinds of fluorescence over time.

The kinetics of the uptake and endosome acidification varied according to a cell, and the special formed cell that did not show the acidification was observed.

We succeeded in time-lapse imaging of endosome acidification, and were able to observe the fluorescence change due to endosome acidification quantitatively and sequentially. The variations of the cell function such as phagocytosis and endosome acidification might be important to understand the life phenomenon.

(COI: No)

P1-286

Intracellular dynamics of estrogen-related receptors

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Estrogen-related receptor (ERR) is a member of the nuclear receptor superfamily and has highly homology with estrogen receptor (ER) α. They have three subtypes (α, β, and γ) that are widely expressed throughout the body including placenta, uterus, and brain. Although endogenous ligands of ERRs have not been identified to date, ERRs regulate the transcription of target gene by their constitutively active structure. The transactivity of ERRs is repressed by binding with diethylstilbestrol (DES), a potent synthetic estrogen. Therapeutic treatment of DES in the pregnancy has been known to be associated with abnormality of reproductive development of the offspring. In this study we analyzed intracellular dynamics of ERRs treated with DES to elucidate molecular mechanism of ERR action. Using live-cell imaging with fluorescent protein labeling, we found that all subtypes of ERRs were mainly localized within the nucleus. Upon DES treatment, the expression of each subtype of ERRs changed from diffuse to punctate pattern in the nucleus. Fluorescent Recovery After Photobleaching (FRAP) analysis revealed the reduction of intranuclear mobility of all subtypes of ERRs after DES treatment. These results show the relationship between the inactivation of ERRs by DES and the cluster formation of ERRs concomitant with mobility reduction. We hypothesize that DES-bound ERRs recruit cofactors to form a protein complex that induces transcriptional repression. Detailed quantification of FRAP analysis has been examined.

(COI: No)

P1-287

Preparation and application to in vivo noninvasive imaging of near-infrared organosilica nanoparticle

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Recently, in vivo imaging with near-infrared fluorescence is used in many fields including molecular imaging. We prepared near-infrared fluorescent nanoparticle (NIR-NP). NIR-NP enables to trace its in vivo behavior through the body. As its property, we can noninvasively observe how nanoparticle reaches the target. NIR-NP was made from mercaptopropyltrimetoxysilane and near infrared fluorescent dye. NIR-NP was evaluated using electron microscopy and in vivo imaging system. We administrated NIR-NPs to mice, then measured the distribution and fluorescent intensity. The accumulations of NIR-NP were detected highly sensitively. Then, we applied NIR-NP to tumor-bearing mice. NIR-NPs were detected in tumor tissue due to EPR effect. In addition, endogenous phagocytes or RAW 264.7, mouse leukemic monocyte macrophage cell line, were labeled with NIR-NP, and they were applied to tumor-bearing mice. Accumulations of the labeled cells to tumor tissue were detected. In addition, biodistribution and intensity of fluorescence were changed and decreased according to time. These results suggested that labeled cells were excreted through a certain pathway. Biodistribution and excretion of labeled cells are unclear. NIR-NP and its application to in vivo imaging are useful to research on the mechanism of the excretion of labeled cells. We believe that understanding of excretion pathway of nanoparticle and labeled cells can be breakthrough of the nanomedicine.

(COI: No)

P1-288

Development of organosilica nanoparticle for photodynamic therapy (PDT) and single cell analysis of PDT effect

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PDT is a local treatment using photochemical reaction of the photosensitizer and excitation light. It has some merits such as less damage to normal tissue, less pain in the treatment. Nanoparticle improve the PDT effect because nanoparticle can target to tumor tissue. We have developed functionalized organosilica nanoparticle for PDT. We performed single cell imaging and quantitative analysis of the cell death to evaluate PDT effect. RAW267.4 cells, a macrophage cell line, were incubated with the particles for PDT overnight. The culture medium containing propidium iodide (PI) was exchanged to detect cell death. The cells were irradiated the excitation light of the ultraviolet lamp. Fluorescence intensities of the particle in all cells and of the PI in killed cells were analyzed. 13 kinds of particles for PDT were evaluated. Some they showed better cytotoxic activity under an excitation wavelength of 650 nm. The single cell imaging and analysis demonstrated time course of cell death and heterogeneous response against PDT. Some cells showed cell death but some didn't be dead in spite of nanoparticle component cell (PDT resistant cell). So cell death wasn't dependent on the quantity of nanoparticle uptake. We speculated that the PDT effect vary by the cellular localization of the nanoparticle. Further experiments will be required to improve PDT effect and to understand the mechanism of PDT resistant.

(COI: No)

P1-289

IL-6 modulates the proinflammatory nature of rat primary cultured microglia

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Accumulatingevidences have shown that neuronal injury affect neuroprotective or neurotoxic actions of microglia by modulating their cytokine and growth factor release. The cytokines and growth factors may affect microglial cells in an autocrine manner. Among the cytokines, interleukin-6 (IL-6), consisting of 184 amino acids, may be one of the most abundantly produced cytokine by activated microglial cells. Yet, the effects of IL-6 on microglial cells are still to be elucidated. To understand how microglia respond to IL-6, we examined the effects of IL-6 on primary cultures of rat microglia where the cells were treated with IL-6 (10 ng /ml) and incubated for various periods. Incubation with IL-6 for 30 min - 5h caused phosphorylation of STATs 1 and 3 as revealed by immunoblotting. After incubation with IL-6 for 2, 5 or 24 h, total RNA samples of the microglial cells were collected for quantitative RT-PCR (qPCR). qPCRshowed that microglial cells increased the expression of mRNA encoding IL-18 but decreased IL-4 at 2 h after IL-6 addition. At 5 h, they increased mRNA encoding IL-1b and IL-18 and suppressed those for IL-4 and IL-10. At 24 h, these changes almost disappeared. Although IL-6 has been recognized as a pleiotropic cytokine, its action on microglial cells appeared to be predominantly proinflammatory. The proinflammatory actions of IL-6 may be mediated by phosphorylated STATs.

(COI: No)

P1-290

Circadian rhythm orchestrates the synaptic homeostasis via microglia

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Circadian rhythms are a 24-hour oscillation process, sustained by a molecular clock and provide a temporal matrix that ensures the coordination of homeostatic processes of animals. Like other immune cells involving the homeostasis, microglia also posses clock genes and in addition to immune surveillance, microglia removes damaged neurons and dysfunctional synapse in brain. To gain insight into possible roles of circadian rhythm in the modification of synaptic structures, we sampled prefrontal brain tissues from rats at ZT0 (Zeitgeber time, light on) and also at ZT12 (lights off). Interestingly, the increased expression of CD68, F4/80, CX3CR1, interferon regulatory factors and Matrix metalloproteinases where as decreased expression of some metabotropic glutamate receptors in the cerebral cortex at ZT0 compared to ZT12. To go further, we focused on primary microglial culture where glutamate stimulates IRF8 and noradrenaline (which peak during active period of circadian cycle) abolishes the glutamate effects. Additionally, glutamate increased the expression of IRF8 that was abolished by NA. Taken together, our data of study on nocturnal mammals provide evidence that the roles of microglia in circadian rhythm; microglial cells eliminate glutamatergic input from the thalamus during the sleep period that is abolished by activated NA neurons during the wake period.

(COI: No)

P1-291

Toll-like receptor 3 mediated activation of microglia; an analysis of its signaling pathway

Takamoto, Masumi; Choudhury, Mohammad E; Islam, Afsana; Kawakami, Ayu; Yano, Hajime; Tanaka, Zyunnya (*Depts. Molecular and Cellular Physiology, and Neurosurgery, Graduate School of Medicine, Ehime University*)

Microglial cells rapidly become activated in response to endogenous ligands for Toll-like receptors (TLRs) that are produced in pathologic brains. The activated microglia are supposed to aggravate neuropathologic processes in various neurological disorders. Therefore, signaling pathways from TLRs responsible for the activation of microglia have been intensively investigated, whereas the majority studies have addressed TLR-mediated MyD88-dependent pathway. In this study, we investigated the response of rat primary microglial cells to poly I:C (pIC), a synthetic TLR3 ligand. TLR3 links to only MyD88-independent pathway. pIC induced iNOS expression and subsequent NO production more weakly than LPS, a ligand for TLR4 that links to both MyD88-dependent and independent pathways. Immunoblotting study showed that pIC induced strong STAT1 phosphorylation and elevated IRF1 expression in microglial cells 3 h after addition of the ligand. LPS weakly caused the similar changes but CpG did not exert any effects. Furthermore, pIC alone induced phosphorylation of MSK1 one h after addition of pIC. Unlike LPS, pIC did not promote NFkappaB translocation into nuclei. However, enhancement of MSK1 phosphorylation might be related to phosphorylation of NFkappaB p65, leading to the binding to the enhancer regions of the target genes. These results suggest a possibility that TLR3 activates microglial cells through a pathway distinct from TLR4-employed one.

(COI: No)

P1-292

Glutamate and noradrenaline modulates phagocytosis of rat primary cultured microglial cells

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Microglial cells have been demonstrated to express a variety of receptors for neurotransmitters. Among the receptors, adrenergic and glutamatergic receptors have been well investigated on their expression and functions. In this study, we have addressed the effects of noradrenalin (NA) and glutamate (Glu) on microglial phagocytic activities with the use of rat primary cultured microglial cells. Microglial cells were cultured in serum-free DMEM containing supplements such as insulin and transferrin. When evaluating their phagocytosis activity, the cells were incubated with PKH26 microparticles possessing red fluorescence for 45 min, followed by fixation and staining with FITC-labeled Phalloidin. The area with red fluorescence within microglial cells was defined as an index for their phagocytic activity. Consequently, microglial cell were found to promote their phagocytic activity in response to Glu, and NA abolished the promoting effect of Glu when NA and Glu was simultaneously added to microglial culture. Na alone did not show any significant effects. Glutamatergic agonists AMPA, NMDA and kainite exerted similar promoting effects to Glu. We have recently found some experimental evidence that microglial cells in the normal and pathologic brains are engaged in the control of synaptic transmission through phagocytic elimination of synapses. Collectively, Glu and NA are involved in the regulation of the synaptic transmission through the control of microglial phagocytic activities.

(COI: No)

P1-293

Expression and interaction between CD38 and TRPM2 in microglia

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CD38 and cyclic ADP-ribose (cADPR) formation have been identified in the hypothalamus and are critical for Oxytocin (OT), but not arginine vasopressin (AVP) secretion, with profound consequential changes in social behaviors in mice. However, expression and role of CD38 in glial cells still remains elusive. In the present study, we examined the immunolocalization of CD38 and TRPM2 which was reported to bind to CD38. In the hypothalamus, CD38 immunoreactivity was found more commonly in OT neurons than AVP neurons. In the CD38-deficient hypothalamus and posterior, stronger staining of OT was observed, suggesting accumulation of OT due to lack of the releasing process, as reported previously. Co-expression of CD38 with glial cells showed that CD38 was rarely expressed in glial fibrillary acidic protein (GFAP)-positive astrocytes. However, expression of CD38 protein in microglia was detected and more expression of CD38 in microglia was observed in the lipopolysaccharide-injected mouse brain. The up-regulation of CD38 was also observed in primary cultured microglia. Expression of TRPM2 was also confirmed and partially merged with CD38. Knocking down of TRPM2 significantly changed the expression level of CD38. These results suggest that CD38 and TRPM2 interact each other and may play an important role in microglial function.

(COI: No)

P1-294

Inhibitory effects of noradrenaline and a hypnotic bromvalerylurea on LPS-induced proinflammatory activation of microglia

Kawakami, Ayu; Ishii, Yurika; Takamoto, Masumi; Choudhury, Mohammad E; Islam, Afsana; Yano, Hajime; Tanaka, Junya (*Dept. Molecular and Cellular Physiology, Graduate School of Medicine, Ehime University.*)

Activated microglia in the pathologic brain presumably aggravate neuropathological processes by releasing potentially neurotoxic substances such as proinflammatory cytokines and reactive oxygen/nitrogen species. Therefore, some intervention suppressing the aggravating activation in an appropriate manner, it would be a promising novel treatment for the brain diseases. In this study, we compared inhibitory effects of noradrenaline and a hypnotic bromvalerylurea (BU), both of which can inhibit LPS-induced NO release by rat primary cultured microglial cells to the similar extent. Noradrenaline (NA) suppressed LPS-induced nuclear translocation of NFkappaB and subsequent STAT1 phosphorylation. However, NA did not inhibit IL-6-induced STAT1 phosphorylation. By contrast, BU did not suppress NFkappaB translocation, but inhibited STAT1 phosphorylation. Simultaneous addition of NA and BU to LPS-treated microglial cells caused multiplier inhibitory effects on NO-release. Although these results suggest that NA and BU inhibit activated microglial cells through distinct manners, it is not yet well elucidated the mechanisms underlying inhibitory actions of BU. The suppressive action of BU may depend on its on JAK1, because BU suppresses interferon-gamma-induced STAT1 phosphorylation in peripheral macrophages. We are currently conducting studies to elucidate the distinctions between NA and BU actions more clearly.

(COI: No)

P1-295

Noradrenalin suppresses proinflammatory reactions of LPS-treated microglial cell

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Noradrenaline (NA) has been well-known of its anti-inflammatory effects on LPS-terated microglial cells. The aim of this study was to elucidate the mechanisms underlying the suppressive NA effects using rat primary cultured microglial cells. NA, an a1 agonist, phenylephrine (Phe) and a b2 agonist, terbutaline (Ter) suppressed LPS-induced elevated expression of mRNA encoding inducible nitric oxide synthase (iNOS) and other proinflammatory mediators by rat primary microglia. Both an a1-selective blocker terazocine and a b2-selective blocker butoxamine overcame the suppressive effects of NA. NA prevented LPS-induced translocation of NFkB into nuclei. LPS decreased IkB followed by phosphorylation of signal transducer and activator of transcription 1 (STAT1) and elevated expression of interferon regulatory factors (IRFs) 1 and 8. NAinhibited LPS-induced these changes. When NFkB expression was knocked down with siRNA, LPS-induced STAT1 phosphorylation and upregulated IRF1 expression was largely abolished. NA did not suppress IL-6 induced STAT1 phosphorylation. These results suggested that one of the critical mechanisms underlying the anti-inflammatory effects of NA may be the inhibition of NFkB translocation. Since NA, Phe and Ter exerted almost the same effects and H89 did not show significant antagonistic effects, the suppressive effects of NA might not be dependent on specific adrenergic receptors and cAMP-dependent signaling pathway.

(COI: No)

P1-296

Effects of IL-18 on rat mixed glial cell culture

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Reactive phenotypes of glial cells including astroglia, oligodendrocyte progenitor cells or NG2 glia, and microglia have been repeatedly documented in the ischemic penumbra of the stroke brain. The reactive phenotypes may be induced by some diffusible factors from the ischemic core lesions, where many bone marrow-derived macrophages accumulate. We have addressed interleukin-18 (IL-18) among the many kinds of factors produced in the ischemic core of rat brains, whose middle cerebral artery was transiently occluded for 90 min. Although IL-18 is quite abundantly produced in the ischemic core, it is still to be elucidated what kinds of roles IL-18 play, what kinds of cells are the targets of IL-18, or how IL-18 works in the ischemic brain. To solve these questions, we have investigated the effects of rat recombinant IL-18 on rat primary mixed glial culture. The culture was started from the whole forebrains of neonatal rats and maintained for 10-13 days. Then, the culture was incubated with IL-18 (0, 1, 5, 25 ng/ml) for 24 h followed by fixation for immunocytochemical staining, collection of protein and total RNA samples, for immunoblotting and RT-PCR, respectively. In response to IL-18, mRNA expression that encodes type I interferon, nestin, NG2, olig2 and hepatocyte growth factor (HGF) was increased in a dose-dependent manner. These results suggest a possibility that IL-18 may be responsible for activation and increase in the number of oligodendrocyte progenitor cells.

(COI: No)

P1-297

Na^+/H^+ exchanger isoform 1 is involved in regulation of microglial cell volume

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Severity of brain edema is one of the critical factors to determine the outcome of stroke patients. Na+/H+ exchanger isoform 1 (NHE1) has been implicated in homeostasis of cell volume by introducing Na+ into the cytoplasm that lead to cell swelling. We have shown that treadmill exercise during acute phase after transient middle cerebral artery occlusion ameliorated brain edema that accompanied downregulated NHE1 expression. The treadmill exercise increased blood corticosterone concentration and the administration of anti-glucocorticoid and anti-mineralocorticoid agents abolished favorable effects of exercise. In this study, we have addressed the effects of NHE1 expression on glial cell volumes, because there should be almost no viable neurons in the ischemic brain tissues. Incubation of rat primary mixed glial cell culture with low concentrations of corticosterone lead to decreased NHE1 expression and showed resistance to incubation with culture medium diluted with water as revealed by LDH assay. Immunocytochemical staining demonstrated that microglial cells expressed NHE1 the most remarkably in the mixed glial cell culture. NHE inhibitors reduced individual cell size in pure microglial cell culture based on cell area determination of microglial cells that had been stained with Hoechst 33258 and FITC-labeled phalloidin. These results suggest that inhibition of NHE1 activities in microglial cells in the ischemic brain may lead to amelioration outcomes of stroke.

(COI: No)

P1-298

Activated microglia in the substantia nigra pars reticulata and globus pallidus of rats with 6-OHDA-induced Parkinsonism

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Activation of microglial cells in the substantia nigra pars compacta (SNpc) and the globus pallidus in a 6-hydroxy dopamine (6-OHDA)-induced rat Parkinsonism model. In this study, we have conducted immunohistochemical analyses on activation of microglial cells. Microglial cell activation was observed not only in the SNpc but also the SN pars reticulata (SNpr), where no neuronal death was observed. Microglial cells in the SNpr characteristically expressed CD68, a marker for phagocytes, suggesting that the cells are engaged in phagocytosis. Immunoreactivity of glutamate receptors (mGluR1, NMDAR2D) as well as PSD95 (a marker for post-synapse) was reduced in the region of the SNpr where activated microglia were markedly accumulated. Immunoblotting study using anti-synaptophysin antibody showed the decrease of synapses in the SN. Furthermore, quantitative real-time RT-PCR demonstrated the decrease in the level of mRNAs encoding glutamate receptors, NR2D (one typ2 of NMDA receptors), mGluR1 and mGluR4. Rat primary microglial cells incubated with glutamate enhanced phagocytic activity by microglial cells. These results suggest that disinhibited glutamatergic neurons in the subthalamic nuclei caused microglial in the SNpr and GP. Then, activated microglia may be engaged in elimination of glutamate receptors in the SNpr and GP, leading to the suppressed activity of GABArgic neurons. Microglia may partially suppress the effect of DArgic neuronal degeneration on the motor symptoms of Parkinsonism.

(COI: No)

P1-299

Treadmill exercise after middle cerebral artery occlusion ameliorates brain edema

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Rehabilitation may be the most effective therapy for stroke, yet mechanisms underlying the curative effects are to be elucidated. In this study, the effect of treadmill exercise on ischemic brain edema was investigated. Wistar rats were subjected to transient (90min) middle cerebral artery occlusion (MCAO). The area of the lesion was measured with magnetic resonance imaging (MRI) on 1 day-post reperfusion (1dpr), and only rats with substantially large ischemic lesion were grouped into exercise and non-exercise ones. Treadmill speed was at 4 - 6m/sec and the rats ran only for 10 min/day at 2, 3, and 4 dpr. On the 5dpr, the brain lesions were again examined with MRI. Consequently, the exercise significantly reduced brain edema and ameliorated the motor function that was evaluated one month after MCAO. The ameliorating effect of the exercise was abolished when anti-glucocorticoid agent mifepristone or anti-mineralocorticoid agent spironolactone. Orally administered low dose of corticosterone suppressed the brain edema in the non-exercise group rats. As revealed by quantitative real-time RT-PCR, the exercise prevented the elevation of mRNA encoding aquaporin 4 (AQP4) and Na+/H+ exchangers (NHEs). These results suggest that the treadmill exercise increases glucocorticoid level in the circulation, leading to suppression of AQP4 and NHEs expression that results in the amelioration of brain edema.

(COI: No)

P1-300

Early-life stressed mice easily induced the epilepsy by application of pentylenetetrazole

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Epilepsy is potentially triggered by neuronal remodeling. For example, activity of newly formed synapses in CA3 or CA1 becomes a cause of epilepsy (Ben-Ari et al., 2008). Previously reported, decrease in stability of mushroom spines in somatosensory cortex and hypersensitivity is detected in early-life stressed mice (Takaturu et al., 2009). This model also showed the increase in growth of mushroom spines which potentially compensate the loss of spines. Thus, this early-life stressed mice possibly be suffered the epilepsy more easy compared with those in control mice. In this study, we prepared the maternal deprivation (MD) mice as follows. MD mice were separated from their mother from post-natal day 2 (P2) to P14, for 3 h every day. The separated MD mice were placed isolated from one another in a locally-made incubator with regulated humidity and temperature. Then we injected pentylenetetrazole (PTZ; 20mg/kg or 30mg/kg) intraperitoneally every other day in adulthood (P56-84) for a month. After injection, we observed the behavior of mice for 30 minutes in the home cage. To estimate behavioral scale, we use Racine scale (RS) (Racine et al., 1972). The behavioral test showed that high RS was recorded in MD mice in early phase after starting to injection compared with those in control mice. This result indicated that MD mice may be vulnerable to epilepsy more than control mice. We are going to observe the concentration of glutamate and GABA in hippocampus by in vivo microdialysis and investigate the mechanism of epilepsy by using molecular biological experiment.

(COI: No)

P1-301

Homeostasis of glutamatergic synapses is disrupted by early-life-stress

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Stress during early life stage induces several neuropsychological disorders in adulthood. The disorders which related with early-life stress should be induced by functional alteration of the glutamatergic system. However, their underlying mechanisms have not yet been fully understood. In this study, we used maternal deprivation (MD) mice as an early-life-stress model, and studied the changes in the glutamatergic system in adulthood. The glutamate concentration and neuronal activity in the somatosensory cortex (SSC) increased under basal condition in MD mice compared with those in control mice. Stressful physical stimulation (SPS) increased the concentration of corticosterone in the control mouse SSC, but not that of glutamate even under the application of SPS. On the other hand, in the MD mice, although the basal concentration of corticosterone in the SSC was increased, no SPS-induced increase was observed. On the other hand, the concentration of glutamate extremely increased during SPS. It was significantly high for 30 min after stimulation. The expression level of α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid / N-methyl-D-aspartate receptor in the MD mice was also changed compared with those in control mice after SPS. These findings indicate that early-life stress disrupts the homeostasis of glutamatergic synapses.

(COI: No)

P1-302

Odorant X-induced analgesia is not stress-induced analgesia in mice

Yamaguchi, Ran[1]; Ishikawa, Sodemi[2]; Tashiro, Shogo[2,3]; Kajiya, Katsuko[1]; Kanmura, Yuichi[3]; Kuwaki, Tomoyuki[2]; Kashiwadani, Hideki[2] (*[1]Dept Biochem Sci Tech, Facul Agri, Kagoshima Univ, Kagoshima, Japan; [2]Dept Physiol, Grad Sch Med Dent Sciences, Kagoshima Univ, Kagoshima, Japan; [3]Dept Anesthesiol, Grad Sch Med Dent Sciences, Kagoshima Univ, Kagoshima, Japan*)

Previously, we found that an odor molecule (odorant X) showed significant analgesia in mice. The odorant X-induced analgesia was not observed in olfactory-deprived mice, indicating that olfactory input evoked by odorant X trigger the analgesia. Furthermore it was not observed in orexin peptide deleted mice or in orexinergic neuron ablated mice. These results indicated that orexinergic transmission is essential for the odorant X-induced analgesia. However, it has not yet revealed whether the odorant X-induced analgesia is one of the stress-induced analgesia or not. To address the issue, we first examined the elevation of plasma ACTH, one of the stress hormones in odor exposed mice. ELISA analyses revealed that the increase of plasma ACTH was not observed in odorant X exposed mice but evident in TMT, one of the predator odors triggering the stress-induced analgesia in rodent. Next to examine the aversion to odorant X, we performed odor preference/avoidance test using two-chambered odor exposure apparatus. Time spent exploratory behavior for odorant X-perfused chamber was comparable with that for odorless air-perfused chamber, indicating that mice did not show the aversion to odorant X. These results indicated that odorant X-exposure did not induce the acute stress for mice which could trigger the stress-induced analgesia.

(COI: No)

P1-303

The analgesic effect of odorant-X is concentration-dependent

Nagata, Keiichiro[1,2,3]; Tashiro, Shogo[3]; Kajiya, Katsuko[1]; Kanmura, Yuichi[3]; Kuwaki, Tomoyuki[2]; Kashiwadani, Hideki[2] (*[1]Dept Biochem Sci Tech, Facul Agri, Kagoshima Univ, Kagoshima, Japan; [2]Dept Physiol, Grad, Sch Med Dent Sciences, Kagoshima Univ, kagoshima, Japan; [3]Dept Anesthesiol, Grad Sch Med Dent Sciences, Kagoshima Univ, Kagoshima, Japan*)

Recently, we found that odorant-X(one of the terpenoid) exposure induced analgesic effect in mouse. Because the analgesic effect was not observed in anosmic mouse, the analgesic effect was driven by odorant X-evoked olfactry input. In addition, we found that the analgesic effect was mediated by hypothalamus orexinergic neurons. However, it has not yet been examined the analgesia threshold or the concentration-response relationship of odorant X. To address the question, we measured the thermal pain threshold under several concentrations of odorant X using classical hot plate test(54.5 degree). The analgesic effect of odorant X vaporized from 10% odorant X solution tended to be attenuated compared to that from 100% odorant X solution, however, the analgesic effect was still significant. The gas from 1% solution did not show the significant analgesic effect. These data indicate that the analgesic effect of odorant X exposure depends on the concentration of odorant X. In our presentation, we will also discuss the difference between detection threshold and analgesia threshold of odorant X.

(COI: No)

P1-304

Prolactin reduces maternal behaviour impairment by lack of CIN85

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Cbl-interacting protein of 85 kDa (CIN85) is a scaffold/multi-adaptor protein implicated in the regulation of receptor endocytosis, cell division and the cellular cytoskeleton. Recently, we reported that mice deficient of CIN85 expression show hyperactive phenotypes. As a molecular explanation of this phenotype, the absence of striatal CIN85 causes decreased dopamine receptor endocytosis in striatal neurons in response to dopamine stimulation.

We show here another phenotype besides the hyperactivity of CIN85 knockout (KO) mice that of maternal neglect to the newborns. Even though there is no difference in the number of live births from CIN85 KO homozygote, heterozygote and wild-type mothers, respectively, almost all pups born to CIN85 KO homozygote mothers have died within two days of birth. Moreover, despite of the fact that no defect in the mammary glands of CIN85 KO mother mice was found, milk was not detected in the stomachs of most pups. Importantly, when measuring the plasma levels of prolactin (PRL), we detected significantly decreased PRL levels in CIN85 KO mice compared to heterozygote and wild-type mice. We therefore injected PRL (0.05 μg/g bw/day, ip) to pregnant CIN85 KO mice in mid to the last day of pregnancy. It could partially rescue the defect in maternal behavior of the next generation.

Our findings indicate a loss of CIN85 function leads to a neglect-like behaviour of the next generation due to aberrant dopamine-PRL signaling.

(COI: No)

P1-305

Lack of CIN85 causes impairment of maternal behaviour by disruption of fetal environment

Sairenji, Taku[1]; Takanashi, Yurie[1]; Kaneko, Ryosuke[2]; Shimokawa, Noriaki[1,3]; Koibuchi, Noriyuki[1] (*[1]Dept Intgr Physiol, Med Grad Sch, Gunma Univ, Gunma, Japan; [2]Inst Exp Anim Res, Med Grad Sch, Gunma Univ, Gunma, Japan; [3]Dept Nutr, Takasaki Univ Health and Welfare, Gunma, Japan*)

Cbl-interacting protein of 85 kDa (CIN85) is a scaffold/multi-adaptor protein implicated in the regulation of receptor endocytosis, cell division and the cellular cytoskeleton. Recently, we reported that mice deficient of CIN85 expression show hyperactive phenotypes. As a molecular explanation of this phenotype, the absence of striatal CIN85 causes decreased dopamine receptor endocytosis in striatal neurons in response to dopamine stimulation. We show here another phenotype besides the hyperactivity of CIN85 knockout (KO) mice that of maternal neglect to the newborns. Even though there is no difference in the number of live births from CIN85 KO homozygote, heterozygote and wild-type mothers, respectively, almost all pups born to CIN85 KO homozygote mothers have died within two days of birth. Importantly, when measuring the plasma levels of prolactin (PRL) on delivery day, we detected significantly decreased PRL levels in CIN85 KO mice compared to heterozygote and wild-type mice. We therefore have transferred wild type (WT) embryos into the oviduct of KO mice. As a result, many of the mice that were born in the embryonic transfer were neglected. Our findings indicate a loss of CIN85 function leads to neglect behaviour in the next generation due to aberrant environment in fetal period.

(COI: No)

P1-306

Developmental sex differences of the synaptic input onto tuberoinfundibular dopaminergic neurons

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Dopamine neurons located in the ARC are known as tuberoinfundibular dopaminergic (TIDA) neurons. A part of their functions is to inhibit prolactin (PRL) release from the anterior pituitary as a PRL inhibitory factor. Since the basal activity of neurons are controlled by synaptic inputs and there is a sex difference in the release of PRL, we examined the synaptic inputs onto TIDA neurons. Transgenic mice carrying GFP under the control of the rat tyrosine hydroxylase gene (Matsushita et al, 2002) were used in the present study. We first confirmed that the expression of GFP was reliable marker for TIDA neurons by immunocytochemistry. Next, we investigated that the developmental changes in the synaptic input onto TIDA neurons. In male and female mice at the age of 2 and 4 weeks, whole-cell voltage-clamp techniques in acute slice were applied. TIDA neurons were identified as GFP-positive cells by fluorescence microscopy. The frequency of miniature excitatory postsynaptic current (mEPSC) in female mice at the age 4 weeks was significantly lower compared to that in male mice at the same age. On the other hand, the mean amplitude of mEPSC was not affected between sexes at this age. There were no significant changes in the frequency or the mean amplitude of mEPSC between female and male mice at the age 2 weeks. These results suggest the presence of the sex difference in the controlling mechanism for TIDA neurons.

(COI: No)

P1-307

Postnatal changes of excitatory synaptic inputs in the rat masseter motoneurons

Kajiwara, Risa[1]; Nakamura, Shiro[2]; Mochizuki, Ayako[2]; Nakayama, Kiyomi[2]; Kiyomoto, Masaaki[2]; Inoue, Tomio[2] (*[1]Showa Univ Sch Dent, Tokyo, Japan; [2]Dept Oral Physiol, Showa Univ Sch Dent, Tokyo, Japan*)

Feeding behavior of mammals dramatically changes from suckling to mastication during early postnatal period. The postnatal development of oro-facial structures during this period is accompanied by developmental changes of central neural mechanisms involved in controlling jaw movement. However, whether the synaptic inputs to trigeminal motoneurons change during postnatal development is still unclear. In this study, we examined the developmental changes of miniature excitatory postsynaptic currents (mEPSC) in the rat masseter motoneurons (MMNs) during early postnatal period. Whole-cell patch-clamp recordings were made from dextran tetramethylrhodamine-lysine (DRL)-labeled MMNs obtained from P2-5 (n = 7) and P10-15 (n = 9) Wistar rats. After bath applications of strychnine, SR95531 and tetrodotoxin, mEPSCs were observed in P2-15 MMNs. Subsequent addition of CNQX almost completely abolished the mEPSCs. The amplitude of the mEPSCs significantly increased from 17.0 ± 1.7 pA at P2-5 to 21.1 ± 0.84 pA at P10-15 ($p<0.05$). Furthermore, the decay time constant of the mEPSCs was significantly reduced from 2.9 ± 0.24 ms at P2-5 to 1.6 ± 0.16 ms at P10-15 ($p<0.01$). In contrast, there were no significant differences in the frequency between P2-5 and P10-15. These results demonstrate the developmental increase in mEPSC amplitude and decrease in mEPSC decay time during postnatal ages. It is possible that the postnatal development of the synaptic inputs to the MMNs contributes to the transition from suckling to mastication.

(COI: No)

P1-308

Glycine-activated outward currents in neurons of the hippocampus

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Glycine receptors are widely expressed throughout the central nervous system such as spinal cord, brain stem, but their physiological roles in the brain and their endogenous ligands have not been clearly identified yet. In the hippocampus glycine activates pyramidal cells in the area of CA1 and CA3 (M. Mori, 2002). Extracellular electrical stimulation revealed no glycinergic synaptic responses in the CA3 pyramidal cells. Here we sought for the roles of glycine receptors in the hippocampus, using organotypic rat hippocampal slice culture, prepared from P6 rats. Pressure application of glycine (0.3 mM in an application pipette; 50 μm away from the soma of the neurons studied) activated an outward current in the neurons but not in the glial cells identified by their failure to generate action potentials at a holding potential of -70 mV. The glycine-activated currents were blocked by the bath-perfusion of a glycine receptor antagonist, strychnine. Peak amplitudes of glycine-activated current density in interneurons were larger than those in pyramidal cells (CA3 pyramidal cell, 17.5 ± 3.24 pA/pF, n=5; interneurons, 36.0 ± 13.2 pA/pF, n=5). We found that the variance of the glycine-activated currents density in interneurons was much more than that in CA3 pyramidal cells, suggesting that this significant variation of the glycine-activated currents in interneurons could be derived from diversity of the type of interneurons.

(COI: No)

P1-309

An old hypnotic bromvalerylurea ameliorates 6-hydorxydopamine-induced rat Parkinsonism

Higaki, Hiromi; Em, Choudhury; Afsana, Islam; Kawamoto, Chisato; Takamoto, Masumi; Yano, Hajime; Tanaka, Junya (*Ehime Univ, Ehime, Japan*)

Damaged neurons express damage-associated molecular patterns (DAMPs) such, which can activate microglia to display proinflammatory reactions that further aggravate neuronal damage. Therefore, such vicious cycles should be prevented either by inhibiting neuronal damage or microglial activation. We have attempted to suppress the activation of microglia to ameliorate neuronal damage. Recently bromvalerylurea (BU), an outdated hypnotic/sedative, was found to suppress nitric oxide (NO) release by lipopolysaccharide (LPS)-activated microglial cells in a concentration-dependent manner. Inducible nitric oxide synthase (iNOS) expression by LPS-activated microglial cells was suppressed at mRNA and protein levels as revealed by real-time RT-PCR and immunoblotting. A rat Parkinson's disease (PD) model was induced by administrating 6-OHDA into the right striatum to cause a substantial loss of dopaminergic neurons in the substantia nigrapars compacta. BU dissolved in drinking water was administered to the PD model rats at a dose of 50 mg/kg body weight/day. BU administration prevented dopaminergic neuron loss and microglial activation. As revealed by quantitative real-time RT-PCR revealed, BU suppressed expression of mRNA encoding IRF1, IRF8 and IL-6, all of which may be involved in microglial activation. Furthermore, BU ameliorated motor function of the rats as revealed by Rota-rod test. Thus, BU may be a promising agent for the treatment of PD by suppressing microglial activation.

(COI: No)

P1-310

Single-cell imaging mass spectrometry revealed lower abundance of palmitoleic acid in breast cancer stem cells

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Imaging of biomolecules has brought significant progress in microanatomy. We have developed an imaging mass spectrometry (IMS) protocol that enabled visualization of lipids. Previously, we found abnormal lipid metabolism in human breast cancer lesions. To further characterize particular rare cellular populations, we conducted IMS analyses of individual cells isolated by fluorescence-activated cell sorting. As a target, we focused on breast cancer stem cells (CSCs), which are thought to cause cancer relapse and drug resistance.

We dispersed surgically-resected breast cancer tissues, sorted $CD45^-/CD44^+/CD24^-$ CSCs, and analyzed them by time-of-flight secondary ion mass spectrometry-type IMS. To validate the results, we analyzed bulk cells by liquid chromatography tandem mass spectrometry (LC-MS/MS).

We visualized simultaneously 4 fatty acid species and phosphoric acid in sorted CSCs. Integrated ion intensity of palmitoleic acid was significantly smaller in CSCs as compared with that of $CD45^-/CD44^-/CD24^+$ non-stem cancer cells; the tendency was identical in 2 cases. This finding was supported by the results of LC-MS/MS analysis in 3 cases.

Our novel method successfully showed the distribution of lipids within unique microanatomical components in human clinical specimens. The abnormal lipid metabolism in breast CSCs identified in this study may have a future application as an anti-cancer therapeutic target specific to CSCs.

(COI: No)

P1-311

Possible participation of sodium ion / proton exchanger1 (NHE1) in lymph node metastasis of head and neck squamous cell carcinoma

Nomura, Noriko; Kirino, Yui; Kaminota, Teppei; Kobayashi, Yusuke; Yano, Hajime; Tanaka, Junya (*Dept Molecular and Cellular Physiology, Med, Ehime Univ, Ehime, Japan*)

Metastases of head and neck squamous cell carcinomas to the draining lymph nodes precede to distant metastases, and dramatically affect in the prognosis. Thus, prevention of the lymph node metastases is expected as promising therapeutic target for this disease. We had already established highly effective lymph node metastasis model of squamous cell carcinoma by using human metastatic squamous carcinoma cell line SASL1m. By xenografting this cell line to KSN nude mice tongues, we obtained almost 100% of metastases to the submandibular lymph node which corresponds to the draining lymph node for the tongue. We found protein expression of NHE1 is enhanced in SASL1m cells compared with non-metastatic parental cell line SAS. NHE1 acts as a regulator of intracellular pH by excreting proton by exchanging with sodium ion, and has potential to make tumor microenvironment acidic. Simultaneously, NHE1 plays a role as an anchoring point of actin cytoskeleton to the cellular plasma membrane, and participates in cellular motility, polarity and invasive activity. We are exploring possible roles of upregulation of NHE1 in squamous cell carcinoma metastases to the draining lymph nodes by using stable NHE1 knockdown SASL1m cells. The knockdown cells exhibit reduced metastatic rates to the lymph node in the model system described above, and also severely reduced in vitro invasive activities. We would like to discuss about the possibilities of NHE1 as a therapeutic target of this disease.

(COI: No)

P1-312

The KCNK13 channel current is increased by the activation of either the Gi/o- or the Gq-coupled receptor

Matsubara, Miki[1,2]; Tateyama, Michihiro[2,3]; Kubo, Yoshihiro[2,3] (*[1]Niigata Univ Sch Med, Niigata, Japan; [2]Div Biophys & Neurobiol, Dept Molec Physiol, Natl Inst for Physiol Sci, Okazaki, Japan; [3]Dept Physiol Sci, SOKENDAI, Hayama, Japan*)

The KCNK13 channel, a member of the two-pore-domain potassium channel family, is known to be activated by arachidonic acid and inhibited by halothane. In addition, we have previously observed that the KCNK13 channel is activated by the Gi/o-coupled $GABA_B$ receptors ($GABA_BR$) in mouse cerebellar Purkinje cells, but not in oocytes. Here we examined whether or not the activation of the $GABA_BR$ potentiates the KCNK13 channel in human embryonic kidney 293 cells by patch clamp recording. We confirmed that $GABA_BR$ positively regulates the KCNK13 channel. We also observed that the activation of the Gi/o-coupled muscarinic type2 receptor potentiates the KCNK13 channel and the potentiation is inhibited by pretreatment with Pertussis toxin. Furthermore, we observed that the KCNK13 channel is potentiated by the Gq-coupled metabotropic glutamate receptor type1a or muscarinic type 1 receptor (M_1R). The effect of the M_1R was suppressed by application of the PLC inhibitor (U73122, 10 μM) but not by the infusion of 5 mM BAPTA in pipette solution, suggesting that downstream of PLC, excluding the increase in the intracellular Ca^{2+}, potentiates the KCNK13 channel. These results demonstrate that the KCNK13 channel is positively regulated by either the Gi/o- or the Gq-coupled receptor.

(COI: No)

P1-313

Physiological significance of the novel spliced isoform of two-pore domain K^+ channel $K_{2P}5.1$

Endo, Kyoko; Kurokawa, Natsumi; Nakakura, Sawa; Sato, Aya; Ishii, Mizuki; Kito, Hiroaki; Niwa, Satomi; Fujii, Masanori; Ohya, Susumu (*Dept Pharmacol, Kyoto Pharmaceut Univ, Kyoto, Japan*)

The two-pore domain K^+ (K_{2P}) channel, $K_{2P}5.1$ (also known as TASK-2/KCNK5) is one of the background K^+ conductance, is activated by extra- and intracellular alkalization and contributes to the setting of the resting membrane potential in various types of cells. We recently identified the novel splice variants of $K_{2P}5.1$, $K_{2P}5.1B$ from mammalian spleens. They were lacking the N-terminus of the original $K_{2P}5.1A$, however, conserved the C-terminus, which is essential for the forming of functional dimers. In the human embryonic kidney HEK293 cell heterologous expression system, the cellular distribution of CFP-tagged $K_{2P}5.1A$ and/or YFP-tagged $K_{2P}5.1B$ showed $K_{2P}5.1B$ inhibited the trafficking of $K_{2P}5.1A$ to the plasma membrane. Using a fluorescence imaging system, alkaline pH-induced hyperpolarization by the activation of native human $K_{2P}5.1A$ ($hK_{2P}5.1A$) was significantly suppressed and the influx of Ca^{2+} was simultaneously decreased in $hK_{2P}5.1B$-overexpressing human leukemia K562 cells. Recent researches highlighted the potential role of $K_{2P}5.1$ in the pathogenesis of autoimmune diseases such as rheumatoid arthritis and multiple sclerosis. We also found a significant increase in $K_{2P}5.1$ expression in the splenic $CD4^+$ T-lymphocytes from a mouse model of chemically-induced inflammatory bowel disease. The mRNA splicing mechanisms underlying the transcriptional regulation of $K_{2P}5.1B$ may implicate for a new therapeutic strategy in autoimmune and inflammatory diseases.

(COI: No)

P1-314

Morphological analysis of small intestinal organoids

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In the small intestine, the epithelial cells are regulated its differentiation along crypt-villus axis, and specialization along proximodistal axis. Recently, Sato and Clevers have been established the method of primary mouse small intestinal epithelial tissue culture (organoid). By using the organoid technique, we investigated whether specialization of epithelial cells along proximodistal axis reflect the organoids derived from different region of small intestine. Crypts were isolated from mouse jejunum and ileum segments, and then cultured for several weeks. The organoids from each segment were observed histologically and identified cellular population. The organoids were composed of central cyst structures and surrounding crypt-like structures. In the epithelium, four types of mature cells were presented; enterocytes, goblet cells, enteroendocrine cells and paneth cells. However, it seems that cellular population of organoid differed with small intestinal segment, we need further examination such as ultrastructural observation and analysis of the epithelial function. The author has no financial conflicts of interest to disclose concerning the presentation.

(COI: No)

P1-315

Ephrin-B1 and EphB2 expression in the stratified squamous epithelium of the skin and mucosae

Kohara, Yukari; Ogawa, Kazushige (*Fac. Life Environ. Sci., Osaka Prefect. Univ., Izumisano, Japan*)

Eph receptors and ephrin ligands are membrane proteins that regulate cell adhesion and proliferation. Recently it has shown that EphAs and ephrin-As expressed epidermal keratinocytes modulate proliferation, migration and differentiation (Lin et al., 2012) while expressions of EphBs and ephrin-Bs are almost unknown. We previously reported ephrin-B1 and EphB2 localizations in the stratified squamous epithelium of the nonglandular part of the rodent stomach. In this study, we screened ephrin-B1 and EphB2 expressions in stratified squamous epithelia of diverse tissues and organs (tongue, esophagus, nonglandular part of the stomach, palm, sole, anus, lip in adult ICR mice). RT-PCR analysis showed that all members of EphBs and ephrin-Bs were expressed in all tissues examined. Immunofluorescence staining revealed that (1) ephrin-B1 was expressed in keratinocytes of the basal and spinous layer of the epithelia in the mucosae and skins, (2) ephrin-B1 was highly expressed in thick regions of the epithelia, where connective tissue papillae were developed, (3) ephrin-B1 were expressed higher in keratinocytes of the basal layer adjacent to the top of the papillae in the dorsum of tongue and these cells were Ki67-negative, (4) EphB2 was expressed in keratinocytes of the basal layer clearly in the mucosae such as the esophagus and nonglandular stomach. These findings may indicate that ephrin-B1expression pattern is almost the same among stratified squamous epithelia except for the tongue while EphB2 expression pattern differs between the skin and mucosae.

(COI: No)

P1-316

Prosaposin and its receptors in the kidney

Watanabe, Haruka[1]; Shimokawa, Tetsuya[1]; Nabeka, Hiroaki[1]; Msi, Khan[1]; Doihara, Takuya[1]; Yamamiya, Kimiko[1]; Hamada, Fumihiko[2]; Matsuda, Seiji[1]; Kobayashi, Naoto[3] (*[1]Anat Embryol, Ehime Univ Grad Med., Ehime, Japan; [2]Anat, Oita Univ F Med., Oita, Japan; [3]Education C, Ehime Univ Grad Med., Ehime, Japan*)

Prosaposin (PSAP) is the precursor of saposins A-D. Accumulating documents suggest that PSAP is a trophic factor *in vivo* and *in vitro* that induces the differentiation and prevents the death of a variety of cells. Recently, the interaction of PSAP with polycystic kidney and hepatic disease gene 1 was reported. The two proteins regulate cellular proliferation and apoptosis (Sun et al., 2010). These findings prompted us to further investigate the distribution of PSAP in the kidneys. We generated a specific antibody to PSAP and examined the spatiotemporal distribution of PSAP-immunoreactive (PSAP-IR) cells in the kidney. PSAP-IR cells were rarely observed in the renal glomerulus. Strongly immunoreactive cells were observed in the proximal tubules, while weak immunoreactivity was detected in the distal tubules. The background levels of immunoreactivity were also observed in the proximal tubules; this artificial reaction must be examined carefully.

(COI: No)

P1-317

Prosaposin and its receptors in the spleen

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Prosaposin (PSAP) is a trophic factor and activator of sphingolipid hydrolase in lysosomes. PSAP is the precursor of four small heat-stable glycoproteins called saposins (saposin A-D), which are required for the hydrolysis of a variety of sphingolipids by specific lysosomal hydrolases (O'Brien et al. 1988). PSAP is found in several organs and is secreted into biological fluids such as milk, cerebrospinal fluid, and seminal fluid, suggesting that PSAP serves not only as a precursor for saposins inside lysosomes but also as a secretory protein without undergoing proteolysis (Hiraiwa et al. 1992, 1993). We generated a specific antibody against PSAP and examined the spatiotemporal distribution of PSAP-immunoreactive (PSAP-IR) cells in rat spleen. PSAP-IR cells were distributed in both the red and white pulp of the spleen. To identify PSAP-IR cells, double and triple immunostaining was performed using antibodies against PSAP, CD68, and CD1d. These results suggest that antigen-presenting cells in these lymphatic tissues contain abundant PSAP (Shimokawa et al. 2013). In the present study, we examined the distribution of immunopositive cells for the prosaposin receptors GPR37 and GPR37L1 in rat spleen. Receptor-immunoreactive cells were observed mainly in the red pulp of the spleen.

(COI: No)

P1-318

Molecular basis of intercellular adhesion in mesangial cells

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Mesangial cells provide the mechanical support for glomerular capillaries by generating an inwardly directed counter force and regulating the glomerular wall tension by contraction and relaxation. Interaction between mesangial cells and endothelial cells are often observed by EM. However, molecular basis of intercellular junction between two cells is not fully clarified. In this study, we demonstrate the intercellular adhesion molecules expressed in mesangial cells. Recently, we have found that filamin, one of actin-binding proteins, is expressed in both normal and proliferating mesangial cells. Filamin is located in the whole cell body of mesangial cell. Therefore, filamin staining is useful for visualizing mesangial cells. Capillary endothelial cells is detectable by ICAM-2 staining. N-cadherin and catenins are located at the intercellular junctions between mesangial cells. On the contrary, spot signals for l-afadin at the tips of mesangial processes including filamin are observed beneath the glomerular capillary. Immunoelectron microscopy demonstrates that l-afadin is located at cell-cell contact between mesangial cell and capillary endothelial cell. The signals for those adhesion molecules are dramatically decreased during mesangiolysis induced by injection of Thy1.1 monoclonal antibodies. Proliferating mesangial cells observed in the expanded mesangial area at day 5 after antibody injection do not express N-cadherin, catenins and l-afadin. The data suggest that l-afadin is involved in the heterologous interaction between mesangial cell and capillary endothelial cell.

(COI: No)

P1-319

The role of αSMA in renal fibrosis

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Renal fibrosis is the final common pathway of a wide variety of chronic kidney diseases, irrespective of the initial causes of nephropathy. The key player causing fibrosis is the myofibroblast. TGF-β1 overproduced in kidney transforms fibroblasts to myofibroblasts, which induce type I collagen accumulation (fibrosis). They are morphologically characterized by having αSMA formed the stress fiber. Significance of αSMA expression remains unknown. In the present study, the role of αSMA in renal fibrosis was investigated by transfecting αSMAsiRNA into NRK49F cells (rat renal fibroblasts). Immunofluorescence for αSMA exhibited that the stress fibers of αSMA were formed by TGF-β1, whereas it was not found in the cells transfected αSMAsiRNA (the siRNA cells). Also it was shown by Western blotting that αSMA expression was accelerated by TGF-β1 and it was suppressed in the siRNA cells. In transmission electron microscopy, TGF-β1 was exhibited to induce building up of stress fibers and dilatation of RER, while building up of stress fibers was repressed but dilatation of RER was similarly recognized in the siRNA cells. In culture of NRK49F cells in type I collagen gel, TGF-β1 markedly evoked gel contraction, whereas the siRNA cells were leaded to suppression of TGF-β1-stimulated gel contraction. Accumulation of type I collagen was induced by TGF-β1, and not affected by αSMA knock down. Dilatation of RER may correspond to increased accumulation of type I collagen. In conclusion, it was demonstrated that αSMA stress fibers caused gel contraction, but did not influence accumulation of type I collagen.

(COI: No)

P1-320

Study of protective effect against oxidative stress on rheological dysorder of erythrocyte

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We study to evaluate the effects of iron-induced oxidative stress and the protective effects of dehydroepiandrosterone (DHEA) against oxidative damage on rheological properties of erythrocytes. Human erythrocytes were incubated for 1 hour at 37℃ with 0-2 mM $FeSO_4$ in the presence of ascorbate. For evaluations of erythrocyte membrane damage. Thiol content of membrane proteins were measured by Ellman's method. A cone-plate viscometer, and high-shear rheoscope were used to evaluate the rheological parameters in Fe^{2+}/ascorbate-treated erythrocytes. 1) Fe^{2+}/ascorbate treatment impaired erythrocyte deformability and erythrocyte suspension viscosity, with increasing membrane protein oxidation. 2) DHEA partially prevented Fe-ascorbate-induced deformability impairment and decrease viscosity of erythrocyte suspensions, by reason of decreasing its oxidative damage. DHEA is efficacious in protecting erythrocyte's against iron-mediated oxidative injury, which can be attributed to its potent reductant and radical scavenging abilities.

(COI: No)

P1-321

Simulation of changes in ionic mechanisms underlying contraction of ventricular cells during embryonic development

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The heart develops and gains new functions while continuously pumping blood, and heart abnormalities progress to congenital heart malformations; therefore, the developmental program of the heart, including the expression of the genes responsible for various ionic channels, is likely to be tightly regulated. The quantitative changes in ionic channels, pumps, exchangers, and sarcoplasmic reticulum Ca^{2+} kinetics are responsible for the changes in electrophysiological properties of the developing cardiomyocytes. Previously, we demonstrated that the developmental changes in action potentials of ventricular myocytes were well represented, as Na^+ current (I_{Na}) increased before the disappearance of and funny current (I_f), followed by a 10-fold increase in inward rectifier K^+ current via simulation; briefly, the relative conductances of the 9 components were switched between early embryonic (EE) and late embryonic (LE) values and simulated the 512 combinations of the model. Here, we constructed a model to represent "middle" embryonic (ME) stage of guinea pig ventricular cell on the basis of experimental data. We then shifted relative current densities of the 9 components among EE, ME, and LE stages, in order to compare the changes in excitation-contraction coupling mechanisms with the simulated results when all components are shifted equally between EE and LE stages without assuming ME stage.

(COI: No)

P1-322

Augmentation of Na^+/K^+-ATPase expression by aerobic training in male rat skeletal muscle

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Aerobic exercise facilitates oxidative phosphorylation and glycolysis of skeletal muscle. Thyroid hormone (TH) controls metabolic activity in a wide range of tissue including skeletal muscle. To examine the relationship between aerobic training and thyroid hormone action in the skeletal muscle, we have studied whether TH signaling pathway is activated by training with different intensity. We previously reported that adult male rats received 30 min/day aerobic treadmill training showed the suppression of TSH level, increase of TRβ1 mRNA and protein levels, and augmentation of Na^+/K^+-ATPase β expression by T3. In the present study, we constructed a series of reporter plasmids containing truncated mutants of Na^+/K^+-ATPase β promoter region and showed that the region -466/-378 bp function as a novel thyroid hormone response element (TRE) in L6 myoblast-derived cells. Liquid chemiluminescent DNA pull down assay, which is in vitro DNA-protein binding assay, showed that TRβ1 bound to the Na^+/K^+-ATPase promoter region. Chromatin immunoprecipitation assay in L6 cells showed that TRβ1 bound to the nucleotide sequence containing typical TRE. These results indicate that aerobic training alters TH signaling at least in part, and such TH signaling alteration may contribute metabolic adaptation in skeletal muscle through the alteration of sensitivity of TH-target gene.

(COI: No)

P1-323

Contractile function of reconstituted cardiac tissue is facilitated by mechanical stretch

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In the field of cardiac regenerative medicine, development of cardiac tissue with sufficient contraction is needed. To this aim, we developed a three dimensional cell culture system and explored optimal conditions for vigorous contraction. Cardiac cells obtained from ventricle of neonatal rats were cultured in a ring shaped gel (outer diameter: 16 mm, inner diameter: 8 mm) including collagen. The gel construct was cultured in a CO_2 incubator in static condition for seven days. Subsequently, the gel construct was transferred to a mechanical stretching device installed in the incubator and cultured for additional seven days. We explored optimal conditions by manipulating collagen concentration and frequency of the mechanical stretch. Tissue formation and contraction of the ring culture were observed under light-field microscope. Histological analyses were carried out in hematoxylin-eosin stained- and phalloidin stained-paraffin sections. Intense contraction was correlated with formation of concentric, fibrillar aggregation of cardiac cells. In those tissues that showed intense contraction, long axis of the cells was aligned in circular direction of the ring. Optimal concentration for collagen was about 0.7 mg. In the ring culture underwent 10% stretch at frequency of 0.5 and 1 Hz, spontaneous, macroscopic contraction was evident. In contrast, ring cultures kept in static condition only showed microscopic contraction. This result suggests that contractile function of reconstituted cardiac tissue was facilitated by mechanical stretch.

(COI: No)

P1-324

Relation between the Hemodynamic Macroscopic and the Microscopic Parameters: Model Study

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A model consisted of a circulation model and a ventricular cell model is useful to understand the relation among the cellular characteristics and the circulation properties, however, several scale factors can be found unknown, if we try to construct such model. Specifically, there are very few experimental data for the scale factor that converts cellular contraction force to the heart wall active stress, and the scale factor that converts cellular passive elastic force to the heart wall passive elastic stress. In this research, a circulation model, which consists of a cardiac contraction model, Negroni Lascano 2008 model, a cardiovascular hemodynamics model, Heldt 2008 model, and a left ventricular geometry model, Laplace's law model was used to determine the ranges of the unknown scale factors. To calculate the possible ranges of those parameters, we used the experimental hemodynamics data, which includes several end-diastolic and end-systolic pressure and volume data, and rates of the left ventricular volume change, left ventricular wall thickness change, left ventricular pressure change at the moment of the maximum LV volume change. We also assumed that the left ventricular wall passive elastic stress is negative at the same moment. Finally, we found that the scale factor that converts positive cellular passive elastic force to heart wall passive stress and a proportion of contractile force to the active force lies on small ranges which implies that the reliability of these values are fairly high.

(COI: No)

P1-325

Thiamine pyrophosphate preserved cardiac function against ischemia-reperfusion injury

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Background: Thiamine (vitamin B1) deficiency was recognized as a cause of Beriberi (*Kakke*; a neurological disease and heart failure). Dr. Kanehiro Takaki who founded Jikei University, eliminated Beriberi from the Imperial Japanese Navy with an improved diet (thiamine supplementation). Thiamine pyrophosphate (TPP), a thiamine derivative, is an active form of thiamine. However, the effect of TPP on ischemia/reperfusion (I/R) heart has not been elucidated.
Aim: The present study was to investigate the effect of TPP on cardiac function after I/R injury.
Method: Male Sprague-Dawley rats (around 10 weeks old) were used of this study. Hearts were extracted and quickly put into Langendorff perfusion. A balloon was inserted into the left ventricle to measure left ventricular developed pressure (LVDP) by calculating the difference of systolic and diastolic pressure. After 5 min perfusion of Tyrode's solution with or without 100 μM TPP, the hearts were treated with mild ischemia (20 min global ischemia followed by 30 min reperfusion: mild I/R) or with severe ischemia (40 min global ischemia followed by 60 min reperfusion: severe I/R).
Results: In mild I/R, LVDP after 30 min reperfusion in TPP solution were significantly higher than that in control solution (74.6 ± 7.5 mmHg in TPP, 50.7 ± 3.8 mmHg in control, n=3 each, $p<0.05$). Furthermore, in severe I/R, LVDP after 60 min reperfusion in TPP solution were dramatically higher than that in control (60.9 ± 7.1 mmHg in TPP, 13.2 ± 5.2 mmHg in control, n=5 each, $p<0.001$).
Conclusion: TPP has a cardioprotective effect against I/R injury.

(COI: No)

P1-326

Time course of changes in sympathetic nerve activity and arterial pressure during development of obesity in Zucker fatty rats

Shiwa, Yuki; Yoshimoto, Misa; Okano, Rika; Miki, Kenju (*Dept Physiol, Nara Womens Univ, Nara, Japan*)

Obesity-associated arterial hypertension has been implicated in activation of the sympathetic nervous system, activation of the renin-angiotensin system, and sodium retention. However, there has been lack of direct evidence on the changes in sympathetic nerve activity during the development of obesity. In the present study, potential contribution of sympathetic nerve activity to the development of hypertension was assessed in Zucker fatty rats. Male Zucher fatty (fa/fa) rats were chronically instrumented with bipolar electrodes for measurements of renal (RSNA) and lumbar sympathetic nerve activity (LSNA), and a telemeter was used for measurement of arterial pressure (AP). The time course of changes in AP, heart rate (HR), RSNA, and LSNA were measured continuously and simultaneously from 8 to 12 weeks of age. Body weight progressively increased from 256 ± 2g at 8th weeks and to 428 ± 7g at 12th weeks. AP remained constant around 100mmHg. HR gradually decreased throughout the experimental period. RSNA and LSNA did not appear to increase in association with the increase in body weight over 8-12 weeks of age. These results suggest that the progressive increase in body weight over 4 weeks is not directly related to the changes in RSNA, LSNA and HR, and such that AP in Zucker fatty rats.

(COI: No)

P1-327

Effects of peripheral chemoreceptor denervation on cardiovascular and sympathetic responses to obstructive sleep apnea in conscious rats

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Potential role of peripheral chemoreceptor in regulating responses of systemic arterial pressure, heart rate, and sympathetic outflows to obstructive sleep apnea was studied. Wistar male rats were chronically instrumented with electrodes for measurements of renal (RSNA) and lumbar (LSNA) sympathetic nerve activity, and electroencephalogram, electromyogram, and electrocardiogram and with catheter for measurement of systemic arterial pressure and with a tracheal balloon for induction of apnea. The tracheal balloon was inflated for 40 seconds during non-rapid eye movement sleep. Systemic arterial pressure, RSNA, and LSNA increased after onset of the tracheal balloon inflation while heart rate decreased. Bilateral carotid body resection attenuated significantly the magnitude of the decrease in heart rate observed in the intact rats, however it exerted minor effects on the responses in arterial pressure and RSNA and LSNA to the obstructive sleep apnea. These data suggest that peripheral chemoreceptor may play a critical role in the bradycardia in response to obstructive sleep apnea, and a minor role in the increases in systemic arterial pressure, RSNA and LSNA in conscious rats.

(COI: No)

P1-328

Suppressive effects of estradiol replacement on stress-induced pressor response mediated by renal sympathetic nerve in ovariectomized rats

Nagatomo, Yu; Tazumi, Shoko; Ito, Risa; Yoshimoto, Misa; Takamata, Akira; Morimoto, Keiko (*Dept. Environm. Health, Facult. Human Life & Environm., Nara Women's Univ., Nara, Japan*)

We examined whether chronic estrogen replacement has suppressive effects on psychological stress-induced pressor responses by attenuating the renal sympathetic nerve (RSN) and renin-angiotensin system (RAS) in ovariectomized rats. Female Wistar rats aged 9 wk were ovariectomized. After 4 wk, the rats were assigned either to a placebo-treated (Pla; n=6) group or a group treated with 17 β-estradiol (E2; n=6) subcutaneously implanted with either pellet. Two wk later, the rats were denervated renally and implanted with radiotelemetry devices for blood pressure (BP) and heart rate (HR) measurements. These rats underwent cage-switch stress at 2 wk after the renal sympathetic denervation. The stress elevated the BP and HR rapidly and continuously both in the Pla and E2 groups. However, these responses to the stress were attenuated significantly in the E2 group compared with the Pla group. Simultaneously, the stress induced elevations of plasma renin activity and angiotensin II concentration in Pla group, but not in E2 group. In addition, the renal sympathetic denervation attenuated the pressor response in the Pla group, but not in E2 group. Therefore, the denervation abolished the difference in the pressor responses between the two groups. These results suggest that estrogen replacement attenuates psychological stress-induced pressor response by suppressing RSN-RAS activation in the ovariectomized rat.

(COI: No)

P1-329

Influence of high-cholesterol on arrhythmogenicity in mouse atrium

Takahashi, Masaki; Takanari, Hiroki; Morishima, Masaki; Ono, Katsushige (*Dept Pathophysiol, Oita Univ Sch Med*)

Background: Changes in cardiac structures due to inflammation and electrophysiological properties may play an important role to generate chronic atrial fibrillation (Af). High cholesterol (Chol) causes systemic inflammation, however the influence of high-Chol on Af is not clarified. We hypothesized that high-Chol induces fibrosis via inflammation in atrial tissue to ameliorate Af.
Methods: Wild type mice (WT) and mice knocked-out the anti-inflammatory mediator IL-10 (KO) were given normal diet (ND) or high-fat diet (HFD) to organize four groups (WT-ND, WT-HFD, KO-ND, KO-HFD). Body and heart weight, serum Chol level, cardiac function on ultrasound cardiography (UCG), ECG parameters, and duration of Af induced by transesophageal pacing were compared. RNA was obtained from mouse atrium to quantify by real-time PCR.
Results: In both WT and KO mice, HFD increased body weight and serum Chol. UCG revealed no significant differences in cardiac function including left atrial diameter. P wave duration on ECG was significantly longer in KO-HFD than in WT-ND. Af sustained significantly longer in KO-HFD than in other three groups. In KO mice, α-SMA and the K^+ channel significantly increased, where the Na^+ channel decreased. Gap-junctional protein Cx40 significantly decreased in mice given HFD.
Conclusion: Chronic inflammation increases fibrosis, impaires electrical conduction by reducing the Na^+ channel, and shortens refractory period by increasing the K^+ channel. In addition, high-Chol decreases Cx40, which also reduces conduction property in atrium. The combination of chronic inflammation and high-Chol ameliorates Af.

(COI: No)

P1-330

Role of apelin in human atrial tissue with persistent atrial fibrillation

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The aim of this study was to identify a group of genes abnormally expressed in cardiomyocytes with atrial fibrillation (Af), and to examine the role of genes involved in pathogenesis of Af. We analyzed expression profiles of mRNA from patient s right atrial appendage with persistent Af (n=10) and normal sinus rhythm (NSR, n=10) by use of gene microarray platform covering a total of 18855 human genes. We found that 149 mRNAs were differentially expressed between persistent Af and NSR cardiomyocytes, where 6 genes were classified as hormone-related genes. Among them, apelin was significantly highly expressed in Af cardiomyocytes, which was further comfirmed by quantitative real-time PCR. Protein expression of apelin was also markedly increased in Af cardiomyocytes. By exposing Ca^{2+} ionophore A23187, isoproterenol and apelin, neonatal rat cardiomyocytes showed up-regulation of apelin and down-regulation of apelin receptor (APJ). Apelin was highly expressed in cardiomyocytes whereas APJ was highly expressed in fibroblast. Treatment of cardiomyocytes with apelin did not change expression of ion channels ($Ca_V1.2$, KCNJ2, KCNJ3). On the other hands, α-SMA, a marker of myofibroblast, was markedly increased by apelin in a dose-dependent manner. Our data provide first evidence that Ca^{2+} overload and/or β-adrenoceptor stimulation in cardiomyocytes increase expression of apelin which triggers neighboring myofibroblast activation leading to formation of Af substrate in the heart.

(COI: No)

P1-331

Subtype-specific down-regulation of Ca^{2+} and cAMP signaling proteins in diabetic mouse atria

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Diabetes mellitus (DM) is one of high risk factors for atrial fibrillation (AF) and its prevalence in aged population. Atrial structural and electrical remodeling underlies progression of AF. Electrical remodeling is characterized by refractoriness due to a shortening of APD in atrial myocytes. In addition to K^+ channels, Ca^{2+} handling and β-adrenergic receptor (β-AR)/cAMP signaling proteins are major determinants of APD. In order to clarify the mechanism linking DM and AF, we examined the age-dependent changes in expression levels of Ca^{2+} and cAMP signaling proteins in atria of DM mice.

Methods: DM was induced in C57BL/6J mice (male, 8-week-old) by a single injection of streptozotocin (STZ, 180 mg/kg, i.p.). Hyperglycemia was confirmed by a blood glucose test. Four weeks after injection of STZ, hearts were excised from mice under deep anesthesia. Gene expression levels were quantified by qRT-PCR.

Results & Discussion: In atria of DM mice, mRNA levels of L-type Ca^{2+} channel CaV1.2 and CaV1.3, junctophilin-2 (JPH2), SERCA2, adenylate cyclase 6 were significantly lower than vehicle controls (n=6). The level of ryanodine receptor 2 (RyR2) was also lower in DM mice. In contrast, gene expression levels of βAR1 and βAR2 tended to be higher in atria of DM mice than controls, while mRNA levels of βAR3, M2R, AC1, AC5, and IP_3R2 were not different between the two groups. These results indicate that DM causes subtype-specific down-regulation of Ca^{2+} and cAMP signaling proteins, which may lead to a shortening of APD and raise the risk for AF.

(COI: No)

P1-332

Comparison of effects of eugenol on respiratory activity in the brainstem-spinal cord preparation from newborn rat and in the arterially perfused preparation from juvenile ratjuvenile rat

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Eugenol is contained in several plants including clove and modulates neuronal activity through actions on voltage-gated ionic channels and/or on transient receptor potential channels. We have reported effects of eugenol on respiratory rhythm generation in the *in vitro* brainstem-spinal cord preparation from newborn rat (P0-P3). Here we compared effects of eugenol in the *in vitro* preparation with those in the decerebrate and arterially perfused *in situ* preparation from juvenile rat (P12 - P15). In the *in vitro* preparation from newborn rats, bath application of eugenol (0.5-1 mM) decreased respiratory rate accompanied with inhibition of pre-inspiratory neuron burst. After washed out, respiratory rhythm gradually recovered but the duration of inspiratory burst was extremely shortened and this continued for more than 1 hr after washout. In contrast, in the in situ preparation, eugenol (1 mM) induced gradual decrease in the amplitude (but not the rate) of integrated phrenic nerve activity followed by complete disappearance within 10 min. Phrenic nerve activity gradually recovered at 25-30 min after washout with burst duration similar to control values. Thus, we found noticeable difference in effects induced by eugenol between two types of preparations; shortening of burst duration in the *in vitro* preparation but decrease of burst amplitude in the*in situ* preparation. We discuss possible mechanisms of these different effects.

(COI: No)

P1-333

Interaction of testosterone with thyroid hormone on sex differentiation of brain in PD7 female rat pups

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Sex differentiation of brain is determined by sex steroids during the perinatal critical period, ED18 - PD5, in the rat. However, it is still unknown what regulates the steroid sensitivity. In this study, we examined the interactive effects of testosterone (T) and thyroid hormone (T3) in PD7 female rat pups, after ceasing the critical period, on adult ovarian functions and sexual behaviors. Three groups of females were treated with one of following combinations, TP+T3: T propionate (TP, 250 μg / 0.05 mL sesame oil, sc) and T3 (30 μg / 500 nL saline, iv), TP+S: TP and saline, and O+T3: sesame oil and T3. After maturation, observations of vaginal smear revealed that PD7 TP treatment (TP+T3 and TP+S) induces acyclicity. At 9 weeks old, all females were ovariectomized and implanted sc with a T capsule, and weekly tested for olfactory preference (estrous and male odors) and male sexual behavior 3 times. TP+T3 and TP+S, but not O+T3, females showed frequent mount, whereas only TP+T3 females showed masculinized preference, i.e. preferring estrous odor to male odor. After intervening 4 weeks following removal of T capsules, female sexual behavior was tested under estrogen and progesterone priming. TP+S and O+T3 females showed almost 100% lordosis quotient (LQ = # lordoses / # mounts x 100), while TP+T3 females had significantly lower LQ. These demonstrate that T3 is involved in both masculinization and defeminization via the interaction with androgen. We suggest that T3 is one of determinants opening the critical window for brain sex differentiation.

(COI: No)

P1-334

Oxidative stress and anti-oxidative responses during hyperthermia in mice brain and the effect of daily exercise

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Recent studies have shown that hyperthermia induces inflammatory response in rodents. Hypothalamic damage has long been speculated as the mechanism involved in heat stroke, although there are few evidences. In the present study, we assessed oxidative stress in the brain during hyperthermia in mice. In addition, we examined the effect of daily exercise. Male mice (n=20) were used. One group had a voluntary running wheel in a cage (Ex), the other not (NEx). After the 6-w period, each group had a 3-h exposure at 39.5℃ (H) or remained at the housing temperature (C). At the end of the exposure, the animals were sacrificed, and the basal parts of the brain tissues were obtained. The protein expressions of SOD1/2, UCP2, NF-$\kappa\beta$ and 4-HNE were analyzed by Western blotting. Body temperature in both Ex-H and NEx-H groups became body temperature of >41℃, although the NEx-H group reached the level earlier. SOD2 were much greater in the Ex-C group than in the NEx-C group. In addition, SOD2 in the Ex-H group was greater than that in the Ex-C group. The expression of 4-HNE was smaller in the NEx-H group than in the NEx-C group, but NF-$\kappa\beta$ was greater. Hyperthermia may activate anti-oxidative responses, decreasing 4-HNE, one of reactive oxidative species. However, such change was not observed in the Ex groups. The reason may be greater basal ability of anti-oxidative responses or smaller heat stress due to augmented thermoregulatory ability in the Ex groups.

(COI: No)

P1-335

Roles of lysyl oxdase like factor2 (LOXL2) in the recruitment of TGFβ activities on premetastatic niche formation on lymph node metastasis of squamous carcinoma cells

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TGF β plays multiple roles in a variety of physiological processes such as tissue remodeling, wound healing and reorganization as well as pathological events such as tumor metastases. This cytokine is secreted from many cells in inactive latent form, and be activated on various biological demands. We had already established highly effective lymph node metastasis model of squamous cell carcinoma by using human metastatic squamous carcinoma cell line SASL1m. By xenografting this cell line to KSN nude mice tongues, we obtained almost 100% of metastases to the submandibular lymph node which corresponds to the draining lymph node for the tongue. Simultaneously, we found the formation of premetastatic niches prior to the establishment of macrometastases to tongues in this model. We also found upregualtions of several genes including TGF β and LOXL2 in SASL1m cells by gene expression profiling analyses compared with non-metastatic oral cancer cell ACC2. LOXL2 acts as a deaminase on amino group of lysin residue, and participates in the remodeling of extracellular matrices (ECM) by cross-linking of ECM proteins such as collagen or elastin. We identified the expression of LOXL2 protein and confirmed TGF β activities in SASL1m cell culture media, and are exploring the possible interactions of LOXL2 and the recruitment of TGF β activities on premetastatic niche formations on the squamous carcinoma cell metastases to draining lymph nodes.

(COI: No)

P1-336

Tumor-associated vascular endothelial cells express CD90 in rat experimental gliomas

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We have found specific expression of CD90 by tumor-associated endothelial cells (TECs) in experimental GBs (EGBs) that had been prepared by transplanting C6 glioma cells into the rat forebrains. Vasculatures in the normal brain parenchyma are surrounded by astrocytic endfeet, whereas those in the EGBs were not. CD90+ cells in the EGBs expressed an endothelial marker von Willebrand factor (vWF) and NG2 chondroitin sulfate proteoglycan (NG2). herefore, the CD90+ cells were judged as TECs. To elucidate the origin of the CD90+ TECs, C6 glioma cells were transplanted into the forebrain of the rats that had been subjected to bone marrow transplantation with the use of green rat marrows as donors. As a result, only a few number of EGFP+/CD90+ cells were found in the EGB mass. Furthermore, there was a very few number of CD90+ circulating monocyte-derived cells labeled with intravenously injected red fluorescent Rhodamine 6G in the EGBs. EGBs contained a huge number of tumor associated macrophages (TAMs) and TAMs were located around CD90+ TECs. These results suggest that not many but some CD90+ TECs was from bone marrow derived circulating monocytes. CD90 on activated endothelial cells has been recognized as a counterreceptor for CD11b that is a marker for monocytes and macrophages. Therefore, it is likely that CD90+ TECs may enhance influx of circulating monocytes as progenitors for TAMs.

(COI: No)

P1-337

Degradation rate of tyrosine hydroxylase by ubiquitin-proteasome pathway

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Tyrosine hydroxylase (TH) is the rate-limiting enzyme in catecholamine biosynthesis, and the portion of the enzyme controlling the intracellular stability has been assigned to the N-terminus. We have reported that TH is degraded by the ubiquitin-proteasome pathway and that the phosphorylation of Ser residues in N-terminus plays a critical role in the degradation of the enzyme. However, the mechanism by which the phosphorylation triggers this degradation pathway in the cell remains unknown. In this study, we investigated the role of the phosphorylations of TH in the degradation of this enzyme. The enhancement of proteasome activity with USP14 inhibitor decreased the quantity of TH phosphorylated at Ser19 in PC12D cells, although it did not decrease that of TH molecules and that of TH phosphorylated at Ser31 and Ser40. The inhibition of proteasome activity with MG132 increased only the quantity of TH phosphorylated at Ser19 by 5-folds for 8 hr. Moreover, we revealed that TH phosphorylated at Ser19 accounted for about 2% of all TH molecules. Therefore, we propose that the phosphorylation of Ser19 is a trigger for the proteosomal degradation and that TH molecule is degraded at a rate of about 1% per hr in PC12D cells. These results present critical information to understand the mechanism that the level of TH is maintained constant in the cells. Support contributed by: KAKENHI (25461296)

(COI: No)

P1-338

Degradation of anillin in cell cycle is mediated by proteasome

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Anillin, a scaffold protein linking actin and myosin, controls the contractile ring spatially and is required for cytokinesis. Anillin is a substrate of anaphase-promoting complex/cyclosome (APC/C) which acts as an ubiquitin ligase. Therefore, anillin level decreases in the exited cells from M phase to G1 phase. Degradation of anillin is known to be important for cell function in G1 phase beyond cytokinesis because untimely accumulation of anillin in G1 phase is toxic to the cells. Anillin is degraded through ubiquitin dependent pathway, however, it is unclear whether anillin is degraded by proteasome or lysosome.

To investigate the degradation pathway of anillin in cell cycle, HeLa S3 cells were synchronized by a double thymidine block, and treated with proteasome inhibitor MG132 or lysosome inhibitor chloroquine.

Anillin was degraded in cell cycle-dependent manner with chloroquine treatment. On the other hand, MG132 treatment induced accumulation of anillin. These findings indicate that degradation of anillin in cell cycle is mediated by proteasome.

(COI: No)

P1-339

A novel angiogenic agent COA-Cl induced PGC-1α-mediated VEGF production in cultured human fibroblasts

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COA-Cl is a recently developed pro-angiogenic agent. It induces robust tube formation in human umbilical vein endothelial cells. A direct endothelial effect, which is partly mediated by S1P receptor, contributes to angiogenesis by COA-Cl. However, the angiogenic effect of COA-Cl was augmented, when co-cultured with fibroblasts. Therefore, mechanisms by which COA-Cl promotes angiogenesis still remain elusive. Here we addressed the hypothesis that COA-Cl induces VEGF production in fibroblast. COA-Cl (100 μM, 48 h) increased basal secretion of VEGF into culture medium from 16 ± 15 to 274 ± 52 pg/mL ($p<0.05$) in human dermal fibroblasts, as evaluated by ELISA. COA-Cl also up-regulated the expression of VEGF mRNA by 2.1 fold (RT-PCR, $p<0.05$). Two transcriptional regulatory proteins, PGC-1 α and HIF-1 α, have been identified as major activators of VEGF gene. COA-Cl markedly up-regulated the mRNA expression of PGC-1 α, but not HIF-1 α, in a time- and dose-dependent manner. Conversely, siRNA-mediated silencing of PGC-1 α gene attenuated COA-Cl-induced VEGF production. The results suggest that COA-Cl induced VEGF production by up-regulating PGC-1 α in fibroblasts, which underlies the augmented angiogenic effect of COA-Cl in the presence of fibroblast.

(COI: No)

P1-340

Anti-inflammatory actions of an old hypnotic bromvalerylurea on microglia/macrophages

Kawamoto, Chisato; Higaki, Hiromi; Takamoto, Masumi; Choudhury, Emamussalehin; Islam, Afsana; Yano, Hajime; Kawakami, Ayu (*Dept Mol Cell Physiol, Gradu Sch Med, Ehime Univ, Toon, Japan*)

We have recently noticed that an old hypnotic bromvalerylurea (BU) has an anti-inflammatory effect on microglia/macrophages, as BU suppressed expression of pro-inflammatory mediators by LPS-treated rat primary cultured microglial cells or peritoneal/alveolar macrophages. So far there are no literatures on the anti-inflammatory actions of BU, therefore, we aimed to elucidate the molecular mechanisms underlying the BU actions. When treated with interferon-gamma (IFN-gamma), phosphorylation of STAT1 was observed in macrophages that was abolished by BU. BU also suppressed IFN-gamma-induced upregulated mRNA expression for STAT1, IRF1 and iNOS. Similarly, BU suppressed LPS-induced upregulated expression of mRNA encoding IL-1beta, IL-6 and Cox2. In spite of the marked immunosuppressive effects of BU, BU did not suppress LPS-induced nuclear translocation of NFkappaB. ChIP assay revealed that BU did not prevent binding of NFkappaB to the target DNA region. BU suppressed IFN-gamma-induced STAT1 phosphorylation in microglial cells that was incubated with LPS for 3 h. When JAK1 or STAT1 expression is knocked down with siRNA, response of macrophages to LPS was almost disappeared. BU did not affect GM-CSF-induced JAK2-dependent STAT5 phosphorylation in macrophages. These results suggest that BU exert its immunosuppressive or anti-inflammatory effects at least partly through specific inhibition of JAK1.

(COI: No)

P1-341

Therapeutic effects of an old hypnotic bromvalerylurea on sepsis of rats

Sakurai, Yuko; Kawasaki, Shun; Abe, Naoki; Yano, Hajime; Tanaka, Junya (*Dept Mol Cell Physiol, Gradu Sch Med, Ehime Univ, Toon, Japan*)

Sepsis has a high mortality rate, therefore, a novel effective treatment should be developed. We recently observed marked immunosuppressive effects of an outdated hypnotic drug, bromvalerylurea (BU), on lipopolysaccharide (LPS)-activated microglial cells, resident macrophages in the brain. In this study, it was investigated whether BU treatment ameliorated cecum ligation and puncture (CLP)-induced sepsis by suppressing proinflammatory reactions of alveolar and peritoneal macrophages (AMs and PMs). BU suppressed LPS-induced production of NO, IL-1beta, IL-6, TNFalpha and chemokines by AMs and PMs in vitro. Male Wistar rats were subjected to cecum-ligation and puncture (CLP). Shortly after CLP, septic rats were subjected to subcutaneous injection of BU (twice/day) at a dose of 30 mg/kg body weight/day, which was clinically relevant. BU treatment suppressed lung edema and ameliorated blood gas test data, with decreased mortality rate of the septic rats by approximately 38% 48 hours after CLP. These effects of BU may be attributable to the suppression of accumulation of leukocytes in the lung, vascular permeability enhancement and expression of proinflammatory cytokines by AMs and PMs. Although BU is now seldom used in clinics, it is worth reevaluating as a novel agent for sepsis and related disorders.

(COI: No)

P1-342

Suppressive effects of bromvalerylurea on LPS-treated activated alveolar and peritoneal macrophages

Tajima, Yuichi; Kikuchi, Satoshi; Nishihara, Tasuku; Kawasaki, Shun; Abe, Naoki; Yano, Hajime; Aibiki, Mayuki; Tanaka, Junya (*Dept. Molecular and Cellular Physiology, Graduate School of Medicine, Ehime University*)

Recently, we have found anti-inflammatory actions of an old hypnotic/sedative bromvalerylurea (BU) on LPS-treated microglial cells. BU also suppressed LPS-induced NO release by rat alveolar and peritoneal macrophages. BU inhibited expression of mRNA encoding a wide variety of proinflammatory mediators including proinflammatory cytokines (such as interleukin-1beta (IL-1beta), IL-6, tumor necrosis factor-alpha), chemokines CCLs 2, 3, and 4, and inducible nitric oxide synthase (iNOS) by LPS-treatedalveolar macrophages. These results suggest that the inhibitory action of BU exerts at transcriptional level. However, BU did not prevent LPS-induced translocation of NFkappaB into nuclei as well as LPS-induced IkappaB degradation as observed in the microglial cells culture. BU inhibited interferon-gamma-induced and LPS-induced STAT1 phosphorylation but not GM-CSF-induced JAK2-dependent STAT5 phosphorylation. Noradrenaline suppressed LPS-induced activated reactions of microglia similarly to BU, but mainly through suppressing LPS-induced NFkappB translocation into nuclei. To determine the molecular mechanisms underlying the suppressive effects of BU, we are now conducting the studies on comparison of anti-inflammatory effects of BU and noradrenaline.

(COI: No)

P1-343

Tumor-associated macrophages; involvement of ECM proteins in induction of their M2 phenotype in experimental gliomas in the rat brain

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Dense accumulation of macrophages in gliomas has been correlated to the worse prognosis. Such macrophages in the tumor tissue (tumor-associated macrophages; TAMs) display an alternatively activated or M2 phenotype, characterized with suppressed proinflammatory nature and promoting activity for tumor growth. In this study, we aimed to elucidate the involvement of extracellular matrix (ECM) proteins in the induction of M2 phenotypes of TAMs. An experimental malignant glioma model was established by transplanting C6 glioma cells into neonatal rat forebrains. Rats developed visible tumors in the brain within 4 weeks. TAMs densely accumulated in the tissue mass. C6 glioma cells were found to expressed an ECM protein tenascin C (TNC) at a high level. TAMs in the glioma were isolated from the dissected tumor tissue and they were cultured solely or cocultured with C6 cells. TAMs were also cultured on C6 glioma cell-derived ECMs. As a result, solely cultured TAMs expressed mRNA encoding chemokines CCL2, 3, and 4 as well as IL-1beta at high levels. On the other hand, TAMs cocultured with C6 cells or those cultured on C6 cell-derived ECM displayed suppressed expression of these proinflammatory factors. Furthermore, when cocultured with C6 cells, of which TNC expression was knocked down, expression of the proinflammatory mediators were elevated. These results suggest that C6 glioma cell-derived TNC may be responsible for the polarization of TAMs to M2 phenotype.

(COI: No)

P1-344

Oct-3/4 promotes the drug-resistant phenotype of glioblastoma cells by enhancing expression of ATP binding cassette transporter G2

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Drug resistance of malignant tumor cells is a major obstacle for the efficacy of chemotherapeutic treatment. Various solid tumor cells expressed Oct-3/4, which has been implicated in the malignancy and also in poor prognosis of GBs, but little is known of its involvement in drug resistances of GB. In this study, we tried to elucidate the contribution of Oct-3/4 to drug resistance in GB cells by lactate dehydrogenase assay, poly ADP-ribose polymerase cleavage, and efflux assay of an anti-cancer drug doxorubicin. A drug efflux pump gene responsible for Oct-3/4-induced drug resistance was evaluated by quantitative PCR analysis and knockdown by shRNA. Oct-3/4 decreased the susceptibility to chemotherapeutic drugs by enhancing excretion of drugs through a drug efflux pump gene, ATP binding cassette transporter G2 (ABCG2). Moreover, expression of Oct-3/4 was well correlated to ABCG2 expression in patients with GBs. Collectively, Oct-3/4 may elevate ABCG2 expression leading to acquisition of a drug-resistant phenotype of GB cells. The present study provides evidence that a signaling pathway from Oct-3/4 to ABCG2 is a promising target to prevent GB cells turning into cells with drug-resistant phenotypes.

(COI: No)

P1-345

Olopatadine Inhibits Exocytosis in Rat Peritoneal Mast Cells by Counteracting Membrane Surface Deformation

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Besides its anti-allergic properties as a histamine receptor antagonist, olopatadine exerts mast cell stabilizing properties by inhibiting the release of chemokines. Since olopatadine bears amphiphilic features and is preferentially partitioned into the lipid bilayers of the plasma membrane, it would induce some morphological changes in mast cells and thus affect the process of exocytosis. In the present study, employing the standard patch-clamp whole-cell recording technique, we examined the effects of olopatadine and other anti-allergic drugs on the membrane capacitance (Cm) in rat peritoneal mast cells during exocytosis. Using confocal imaging of a water-soluble fluorescent dye, lucifer yellow, we also examined their effects on the deformation of the plasma membrane. Relatively lower concentrations of olopatadine (1 or 10 μM) did not significantly affect the GTP-γ-S-induced increase in the Cm. However, 100 μM and 1 mM olopatadine almost totally suppressed the increase in the Cm. Additionally, these doses completely washed out the trapping of the dye on the cell surface, indicating that olopatadine counteracted the membrane surface deformation induced by exocytosis. This study provides electrophysiological evidence for the first time that olopatadine dose-dependently inhibits the process of exocytosis in rat peritoneal mast cells. Such mast cell stabilizing properties of olopatadine may be ascribable to its counteracting effects on the plasma membrane deformation in degranulating mast cells.

(COI: No)

P1-346

Extracellular Na^+ ion dependency of hypotonic swelling of HeLa cells

Otsuki, Lucia; Kobayashi, Daisuke; Hazama, Akihiro (*Dept Cell Integrative Physiol, Fukushima Medical Univ, Fukushima, Japan*)

The mechanism of cell volume regulation after hypotonic swelling has been widely investigated and the role of ion channels or transporters has been reveiled. On the other hand, the swelling process just after the hypotonic challenge has been considered to be attained by the water influx driven by the difference of osmolarity between extracellular and intracellular solutions. In this study, we examine the possibility of the water influx is enhanced by the ionic influx, especially Na^+ ion. The cell volume of enzymatically suspended HeLa cells was measured by FACS. Hypotonic condition was obtained by the addition of water to the experimental solution. The cell volume was increased rapidly after hypotonic challenge as reported before. The replacement of Na^+ ion by choline suppressed the cell swelling. NKCC inhibitor, bumetanide, inhibited cell swelling by 48% and ENAC inhibitor, amiloride, inhibited cell swelling by 18%. These data suggest that water influx after hypotonic challenge is partially driven by Na^+ ion influx via NKCC or ENAC.

(COI: No)

P1-347

Dysregulation of mitochondrial formyltransferase MTFMT in gastric cancer

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Mitochondrial protein synthesis is responsible for biosynthesis of 13 proteins, which are essential for constitution of mature respiratory chains. Dysregulation of mitochondrial protein synthesis impairs oxidative phosphorylation, which shifts aerobic respiration to anaerobic glycolysis. The enhanced glycolysis, namely Warburg effect, is a hallmark of cancer. However, the molecular mechanism underlying mitochondrial dysfunction in cancer remains unclear. To investigate the regulatory mechanism of mitochondrial protein synthesis in cancer cells, we performed a systemic investigation of the expression level of genes involved in mitochondrial protein synthesis in a large clinical samples derived from patients having gastric cancer. Interesting, MTFMT, a mitochondrial tRNAMet-specific formyltransferase gene, is significantly upregulated in cancer tissues. Because the formyl-tRNAMet is specifically used for start codon ATG dysregulation of MTFMT might have profound effect on mitochondrial protein synthesis. As expected, overexpression of MTFMT in gastric cancer cells decreased mtDNA-derived mitochondrial proteins. Furthermore, the expression of MTFMT was under control of oncogene c-Myc. These results demonstrate that the activation of c-Myc-MTFMT pathway might be responsible for the metabolic changes in gastric cancer.

(COI: No)

P1-348

Identification of the major asynapsis-induced phosphorylation site of mouse HORMAD1

Kikuchi, Yuka; Kogo, Hiroshi; Kogo, Akiko; Sawai, Nobuhiko; Matsuzaki, Toshiyuki (*Gunma Univ. Grad. Sch. Med., Maebashi, Japan*)

HORMAD1 is a mammalian homolog of yeast Hop1, which is necessary for meiotic recombination and surveillance mechanisms. We have made Hormad1 knockout mice, and found that HORMAD1 is necessary for synapsis and synapsis checkpoint in mammalian meiosis. In yeast, DNA double strand break (DSB)-induced phosphorylation of Hop1 is necessary for its function. Mouse HORMAD1 has multiple putative phosphorylation sites, and is intensively phosphorylated in SPO11-deficient meiocytes, where extensive asynapsis occurs due to the absence of DSBs. Despite the expected importance of this asynapsis-induced phosphorylation of HORMAD1, the phosphorylation site has not yet been identified. In this study, we made phospho-specific antibodies against two candidate phosphorylation sites, Ser-307 and Ser-378, and examined their localization on meiotic chromosomes by immunostaining of spermatocyte spreads. As a result, both Ser-307 and of Ser-378 were phospholylated on unsynapsed chromosomal axes at zygotene stage, and on the XY chromosome axes at pachytene stage in wild-type spermatocytes. In addition, interestingly, Ser-307, but not Ser-378, was phosphorylated on entire unsynapsed axes in SPO11-deficient spermatocytes. We further confirmed by western blotting that the band of phosphorylated HORMAD1 was positive for the Ser-307 phosphorylation in SPO11-deficient testes. These results for the first time demonstrate that Ser-307 is the major phosphorylation site of mouse HORMAD1 on unsynapsed axes, providing a clue to reveal the molecular basis of asynapsis surveillance mechanism.

(COI: No)

P1-349

The progeny of bone marrow stem cells with metabolic memory perturbs skin homeostasis

Okamoto, Naoki[1]; Okano, Junko[1]; Kojima, Hideto[2]; Katagi, Miwako[2]; Nakae, Yuki[2]; Terashima, Tomoya[2]; Udagawa, Jun[1] (*[1]Shiga. Univ. Med. Sci., Shiga, Japan; [2]Shiga. Univ. Med. Sci., Shiga, Japan*)

The major cell population in epidermis is keratinocytes with more than 95%, while other cells such as melanocytes and Langerhans cells (LCs) are observed as minor cell population. Although it has been well known that bone marrow-derived cells (BMDCs) are mobilized to replenish the LC population upon severe inflammation, the dynamic state of BMDCs in skin remains unknown at either steady state or mild alteration of microenvironment. Using ionizing radiation at a relatively low dose for skin (10 Gy), we investigated the role of BMDCs in skin under mild alteration of microenvironment. We transplanted KSL (c-kit+Sca1+Lin-) cells, an early form of hematopoietic stem cells, from GFP reporter mice to irradiated wild-type mice in order to chase the progeny of BMDCs as GFP+ cells. The descendants of KSL cells migrate into the epidermis one month after transplantation. The population was heterogeneous and some of them are ramified with MHCII+EpCAM+Langerin+, indicating Langerhans cells. To pursue the metabolic memory on the progeny of KSL cells migrating into epidermis, we transplanted KSL cells from GFP reporter mice with diabetes mellitus to irradiated wild-type mice. The descendants from KSL cells exposed to hyperglycemia affected homeostasis of skin, indicating that dynamic cells (e.g. BMDCs) as well as static cells (e.g. keratinocytes) play an important role on the maintenance of skin microenvironment.

(COI: No)

P1-350

Species difference in expression and localization of androgen receptor in the suprachiasmatic nuclei of normal and hormone-manipulated adult rats and mice

Nemoto, Jo; Jahan, Mir Rubayet; Isram, Md Nabiul; Kokubu, Keiji; Yanai, Akie; Wroblewski, Greggory; Fujinaga, Ryutaro; Shinoda, Koh (*Yamaguchi Univ. Sch. Med., Div. of Neuroanatomy*)

The suprachiasmatic nucleus (SCN) is a master pacemaker of the CNS, which regulates a wide variety of neural and bodily rhythms. Hormones are considered as critical intrinsic modifiers to the SCN clock directly through regulating expression of numerous SCN genes via their receptors. Although we have recently reported that androgen receptor (AR) expression was higher in males than females and critically more prominent in mice than in rats, detailed localization of AR expression and its hormonal regulation in the SCN remain to be clarified in the two rodents. In the present study, detailed AR distribution was immunohistochemically examined in serial paraformaldehyde-fixed sections of the SCN and compared between hormonally manipulated rats and mice. The current results demonstrated that AR expression in the SCN is upregulated by dihydrotestosterone treatment in both castrated rodents and clarified that enhanced AR expression in the rat SCN is localized to the shell part (output part), making a sharp contrast with prominent AR expression in the core part (input part) of the mouse SCN. Our observation strongly suggests that the species difference in effects of clock-controlled androgen secretion on circadian rhythmicity is attributable to the species difference in expression and localization of AR in the SCN. Androgen might be regarded as a species-dependent "clock-controlled clock modifier".

(COI: No)

P1-351

Circadian Rhythm in Skin

Morohashi, Keita[1]; Okano, Junko[1]; Kojima, Hideto[2]; Terashima, Tomoya[2]; Katagi, Miwako[2]; Nakae, Yuki[2]; Udagawa, Jun[1] (*[1]Shiga. Univ. Med. Sci., Shiga, Japan; [2]Shiga. Univ. Med. Sci., Shiga, Japan*)

Animals evolved endogenous timing clocks called circadian rhythms (~24 hours) to adapt external environment. Circadian rhythm is controlled by core clock genes (Bmal1, Clock, Period1/2/3, and Cryptochrome1/2), among which Bmal1 and Clock act as master regulators. Although biological functions of circadian rhythm are well-studied in the central nervous system as well as various peripheral tissues, its role in skin remains unclear. Here, we report epidermal cells isolated from mice show circadian changes in the gene expression of the core clock genes, and this endogenous rhythm in skin is independent of one in the central nervous system. In addition, we show that transepidermal water loss (TEWL), a reflection of the skin barrier function, rhythmically changes throughout a day. Taken together, these results indicated that circadian rhythm in skin regulated the physiological function of skin.

(COI: No)

P1-352

Effects of oxidative stress on circadian rhythm in vitro MEF from PER2::LUC mouse

Yokota, Aya; Haraguchi, Atsushi; Shinozaki, Ayako; Shibata, Shigenobu (*Laboratory of Physiology and Pharmacology, School of Advanced Science and Engineering, Waseda University, Tokyo Japan*)

Oxidative stress is produced by reactive oxygen species (ROS), which can affect the physiological function of cellular proteins, lipids, nucleic acids and other macromolecular substances. Recent studies have focused on circadian-regulated energy metabolism, redox state, and intracellular ROS in living system and disease such as cancer and metabolic syndrome. However, how the circadian system responds to oxidative stress has not understood completely, and we have to reveal it. To clarify this question, we investigated the relationships between circadian rhythm and oxidative stress (H2O2 0.2 mM) produced at various points (CT 4, 8, 16, 20 and 22) in mouse embryonic fibroblast (MEF) cells from PER2::LUC mouse. We demonstrated that each exposure time point to oxidative stress droved the phase shifts (advance and delay), and shift direction were dependent on exposed timings. In addition, oxidative stress led to cell death in a time-dependent manner, which was consistent with the time-dependent phase shift. Furthermore, we found that H2O2 application caused amplitude reduction at a certain time point. Some kinase inhibitors (PKA inhibitor and CaMKII inhibitor) protected stress-induced reduction of amplitude. It is suggested that oxidative stress may be involved in CaMKII stress response pathway. Thus, the relationship between circadian clock and oxidative stress was strongly suggested, and it is necessary to continue to search for a signaling cascade on the phase shift and amplitude reduction due to oxidative stress.

(COI: No)

P1-353

Effects of aging on the peripheral clock gene expression rhythms

Takatsu, Yuta; Tahara, Yu; Shiraishi, Takuya; Kikuchi, Yosuke; Yamazaki, Mayu; Shibata, Shigenobu (*Laboratory of Physiology and Pharmacology, School of Advanced Science and Engineering, Waseda University, Tokyo, Japan*)

In mammals, aging causes the disruption in sleep-wake cycles and the decline in neural activity rhythms in the SCN, suggesting that these changes are provided by age-related decline in clock gene expression rhythms. In this study, we reported age-related changes in clock gene expression rhythms in peripheral tissues using in vivo imaging system. We observed expression rhythms of PER2, one of the clock genes, in the kidney, liver, and submandibular glands of young and aged mice carrying with PER2::LUCIFERASE knock-in. We focused on the character and speed of the response to the entraining stimuli including light, food, and restraint stress. Regarding to light entrainment, the results demonstrated no differences in amplitude and phase of PER2 in peripheral tissues between young and aged mice, although the decline in amplitude of the behavioral rhythms was observed in aged mice. For the entrainment to restraint stress, there were no differences similarly. However, regarding to food entrainment, the phase of aged mice advanced more quickly than that of young mice only in the submandibular glands. In addition, expression levels of adrenergic receptors in the submandibular glands were reduced in aged mice compared to young mice. Thus, the present findings suggested that, in the submandibular glands of aged mice, food entraining signals became dominant signal corresponded with the reduction in adrenal signal.

(COI: No)

P1-354

A Fluorescence Resonance Energy Transfer Biosensor for TAK1 activity

Takaoka, Saori; Matsuda, Michiyuki; Kamioka, Yuji (*Dept. of Path. and Biol. of Dis., Grad. Sch. of Med., Kyoto Univ*)

The stress-activated protein kinase (SAPK) signaling cascade evokes various cellular responses, such as apoptosis, differentiation and inflammation, under various cellular stresses. Here, we report a fluorescence resonance energy transfer (FRET)-based biosensor that responds to various stresses. A FRET biosensor "3592NES" was found to respond to stress-inducing reagents such as anisomycin, tumor necrosis factor-α, and interloikin1-β. Among various inhibitors for protein kinases, we found that an inhibitor against TGF-β activated kinase 1 (TAK1), 5z-7-oxozeaenol, markedly suppressed the stress-induced increase of FRET ratio in 3592NES-expressing cells. Furthermore, siRNAs against TAK1 also abrogated the stress-induced response of the 3592NES-expressing cells, indicating that 3592NES monitors the TAK1 activity. To examine whether 3592NES can monitor TAK1 activity in physiological contexts, we established Lewis lung carcinoma 3LL cells that stably express 3592NES. When the 3592NES-expressing 3LL cells were co-cultured with macrophages and stimulated with polyinosinic:polycytidylic acid (poly I:C), we observed increase in FRET ratio and induction of apoptosis. We also live-imaged 3592NES-expressing 3LL cells that were implanted subcutaneously into mice by two-photon excitation microscopy. High FRET signal was observed in tumor cells locating the periphery of tumor mass. These observations suggest that 3592NES is a versatile biosensor that monitors stress-induced TAK1 activation during inflammation and tumor development both in vitro and in vivo.

(COI: No)

P1-355

Sex-related difference in exponents of Stevens' power law

Saho, Masumi[1]; Ogiso, Nao[1]; Kuroda, Saya[1]; Tokumaru, Osamu[2];
Eshima, Nobuoki[3]; Harada, Chizuru[4]; Yokoi, Isao[2] (*[1]Sch Nurs, Oita Univ Fac Med, Oita, Japan; [2]Dept Neurophysiol, Oita Univ Fac Med, Oita, Japan; [3]Dept Stat, Oita Univ Fac Med, Oita, Japan; [4]Dept Fund Nurs Sci, Oita Univ Fac Med, Oita, Japan*)

Background: The magnitude of sensation is a power function of the intensity of the stimulus, known as the Stevens' power law. We have reported that the inverse process, "making stimulus intensity matching to a designated magnitude of sensation", is not simply an inverse process with the reciprocal exponent (Nishi et al., 2014). The purpose of this study is to examine possible sex-related differences in exponents of Stevens' power law in those two matching tasks.
Methods: Subjects were instructed to make judgments of the apparent magnitude of sensation of loudness, tone, brightness and angle of the elbow joint (M/F = 30/30). The magnitude of sensation was quantified by matching numbers to sensory stimuli ("matching task"). Inversely, intensity of stimuli was matched to natural numbers (0-100) given ("inverse matching task"). Data were analyzed by a linear mixed-effects model.
Results: For all four modalities, matching tasks and inverse matching tasks obeyed power functions with characteristic exponents ($p<0.001$). Exponents of inverse matching tasks were not reciprocal to those of matching tasks for all modalities. Exponents in males were significantly larger than those in females for brightness both in matching and inverse matching tasks ($p=0.02$).
Conclusions: It is suggested that inverse matching task is not a reciprocal function of matching task. There were significant sex-related differences in exponents in brightness.

(COI: No)

Poster Presentations

Day 2

（March 22, 12:45～14:00）

P2-001～P2-057	Embryology, Regenerative Medicine, Development, Growth, Aging
P2-058～P2-087	Cartilage, Bone, Connective tissue
P2-088～P2-115	Muscle
P2-116～P2-138	Digestion, Digestive system
P2-139～P2-172	Oral physiology, Tooth, Salivary gland
P2-173～P2-188	Blood, Lymph, Immunity
P2-189～P2-246	Circulation
P2-247～P2-256	Respiration
P2-257～P2-269	Urinary organ, Renal function, Urination
P2-270～P2-300	Reproduction, Genital organ
P2-301～P2-336	Endocrine
P2-337～P2-355	Histology
P2-356～P2-365	Physical fitness and sports medicine
P2-366～P2-405	Nutritional and metabolic physiology, Thermoregulation

P2-001

Epigenetic regulation on histone H3K27 is involved in redifferentiation process during leg regeneration in the cricket *Gryllus bimaculatus*

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Hemimetabolous insect such as cricket *Gryllus bimaculatus* has remarkable regenerative capacity. When cricket lost a part of leg, distal missing part of leg is regenerated from blastema, which is a population of proliferating multipotent cells. Blastema cells are dedifferentiated from differentiated cells and redifferentiate to several types of differentiated cells to regenerate the lost part. To know whether gene expressions could be epigenetically changed in the blastema cells, we performed transcriptome analysis on blastema cells. Our analysis showed that expression of *Enhancer of zeste* (*E(z)*), which encodes methyltransferase for histone H3K27, was upregulated in the blastema of regenerating leg compared with normal leg. We analyzed the functions of *E(z)* on regeneration process of cricket leg using RNA interference (RNAi). In the *E(z)*(RNAi) cricket, methylated histone H3K27 was diminished as revealed by immunostaining and an extra leg segment was formed between tibia and tarsus of the regenerated leg. Expression domain of a leg patterning gene *dachshund*, which promotes differentiation to tibia, was expanded in tarsus of the *E(z)*(RNAi) regenerating leg. These results suggest that epigenetic regulation on histone H3K27 is crucial for redifferentiation process during leg regeneration in the cricket.

(COI: No)

P2-002

Postnatal growth of hindlimb bones are restricted by undernutrition during early embryonic period

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Epidemiological studies have revealed that maternal undernutrition increases the risk of cardiovascular diseases, diabetes and osteoporosis. We previously reported that maternal 50% dietary restriction during early pregnancy restricts postnatal growth of hindlimb bones in female offspring. We analyzed the effect of 40% dietary restriction during early pregnancy on the growth of the trunk, femur and tibia in rats. Pregnant Wistar rats were divided into two groups. The undernourished (UN) group underwent 40% dietary restriction from E5.5 to E11.5, whereas the control group was fed AIN-93G ad libitum. In female neonates, the trunk and tibia were significantly longer in the UN group offspring than in controls. However, the length of tibia was smaller in UN offspring than in controls at 16 weeks of age. In the mesenchyme of the posterior limb bud at E13.5, the expression level of Grem1, which contributes to maintain limb outgrowth, significantly decreased in UN offspring than in control offspring. These results suggest that undernutrition during early fetal period affects epigenetics of the presumptive limb area and inhibits the postnatal growth of the tibia.

(COI: No)

P2-003

Comparison of early neuronal developmental stages between human iPSCs-derived neurons and rat primary cultured neurons

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We analyzed neuronal development of hiPSCs-derived neurons (hiPS-Neuron) particularly focusing on their early development stages. We cultured two types of hiPS-Neuron, and compared their development with rat neurons by Dotti·s classification. At 2 days in vitro (DIV), both hiPS-Neuron and rat neurons showed three developmental stages 1 to 3. The percentage of neurons at each stage were different between hiPS-Neuron and rat neurons. Furthermore there were significant decreases in the neurite length and numbers, branching points, and axon length in hiPS-Neuron. It is suggested that hiPS-Neuron extends their neurites more slowly than rat primary cultured neurons. Ineteresstingly the axonal differentiation of iCell neurons (CDI) occurs more slowly than ReproCELL DA neurons and rat neurons. We then double-labeled the neuorns for drebrin and F-actin. We found that the localization patterns of F-actin and drebrin in growth cones of iCell Neuron were similar to those of rat neurons. To test whether there is a difference in the effect on F-actin severing and depolymerization drug Cytochalasin D on the growth cone. Cytochalasin D caused drebrin and F-actin to shift from the transitional zone to the distal edge of growth cone in rat neurons and iCell Neurons. These data suggest that although hiPS-Neuron extends their neurite more slowly, it might be useful for pharmacological evaluation.

(COI: No)

P2-004

Influence of endogenous Akt/β-catenin signaling in hypothalamic differentiation from mouse embryonic stem cells

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Mouse embryonic stem cells (mESCs) are reported to differentiate into Rax^+ hypothalamic progenitor cells when cultured as floating aggregates in a growth factor-free chemically defined medium (Wataya et al., 2008, PNAS). However, we found that proportion of induced Rax^+ cells varies between experimental trials (40-85%). The original study also showed that exogenous activation of Wnt or Akt signaling markedly suppresses hypothalamic differentiation from mESCs, raising the possibility that endogenous Wnt or Akt activity is involved in the variability of Rax^+ cell induction. To test this hypothesis, we conducted differentiation culture in the presence of Wnt or Akt inhibitor. Addition of a Wnt inhibitor (Dkk-1) had no effect on the proportion of Rax^+ cells, but an Akt inhibitor (AktiVIII) moved the proportion into a higher range. Similar effects were produced by a compound (XAV939) that stimulates degradation of β-catenin, a downstream effector for Akt as well as Wnt pathway. Moreover, we found a clear negative correlation between Rax^+ cell proportion and total cell number in the differentiated mESC aggregates. These data indicate that selective inhibition of endogenous Akt/β-catenin signaling enriches Rax^+ hypothalamic progenitors derived from mESCs, possibly by suppressing the proliferation of other lineage cells.

(COI: No)

P2-005

Improvement of the efficiency of differentiation of mouse embryonic stem cells into insulin-producing cells by lentivirus-mediated transduction of transcriptional factors enriched in mouse islets

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Cell replacement therapy for diabetes has become possible by artificially generated pancreatic β-cells from pluripotent stem cells. However, the yield and the functional maturation of insulin-producing cells differentiated from these cells are still poor. It is important to develop a method to facilitate the differentiation efficiency and the maturation of insulin-producing cell to bona fide pancreatic β-cells. In the present study, we compared the gene expression between the terminally differentiated cells and mouse islets by microarray analysis. And we found 86 genes that expressed in islets but not in differentiated cells from mouse embryonic stem cells(mESCs), and six genes including *Mesp1*, *Rfx6* and *Isl1* of them have function as "DNA binding" with Molecular Function Ontology. We examined tissue distribution of the six genes by quantitative PCR analysis and found that the expression of almost of the genes were highest in islets. We next prepared lentivirus carrying each gene and transduced into differentiating mESCs. In Mesp1-transduced cells, some β-cells specific gene expressions were increased compared with normally differentiated cells. *Rfx6* and *Isl1* are known to have important role in pancreatic development, whereas the role of *Mesp1* and other genes in pancreatic development have not been revealed. In conclusion, this study suggests possible candidate for unknown key factors for pancreatic development and differentiation.

(COI: No)

P2-006

Sbno1 is required for maintenance and differentiation of the neural stem cells

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Sbno1 is included in strawberry notch (sbno) family protein, of which structure is similar to nucleic acid helicases. Drosophila mutants of sbno gene exhibit abnormal morphologies, which are common to mutants of Notch signal related genes, suggesting that sbno is involved in Notch signaling pathway. However, knockdown or knockout experiments utilizing other animals, such as nematode and fish, did not clearly indicate sbno function in Notch signaling pathway. Recent genome analysis of human suggested that SBNO1 is relevant to normal development and function of brain. Here, we examined function of Sbno1 in mouse embryogensis focusing on brain development. Our immunohistological observations utilizing anti-Sbno1 antibodies showed strong nuclear expression of the protein in the differentiating neurons, whereas the expression was weak in the zone facing the ventricle. We then constructed floxed Sbno1 transgenic mouse mutant line, and crossed it to Emx1-Cre driver line to achieve dorsal forebrain specific Sbno1 knockout out. The embryonic cortex of the mutant exhibited premature neuronal differentiation and robust cell death. When we observed an earlier stage of cortical development in the mutant embryos, we found ectopic expression of p53, the tumor-suppressor protein. These observations in the mutant suggest that Sbno1 is required for two aspects of stem cell function, cell cycle regulation and production of neurons.

(COI: No)

P2-007

Identification of a novel pluripotency factor that is conserved between planarian neoblasts and human Muse cells by using planarian neoblast-specific antibody

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Muse cells are human adult stem cells that are found in mesenchymal tissues. Muse cells can be differentiated into various types of cells of all three germ layers, and are not tumorigenic. Looking over across the animal kingdom, we can find similar types of multipotent adult stem cells in many evolutionally primitive animals. Among such animals, planarian has been used as a model organism for regeneration. The pluripotent stem cells in planarians are called neoblasts. We hypothesized that the evolutional origin of Muse cells might be neoblasts. Using newly-developed neoblast-specific antibodies (Abs), we performed immunoprecipitation (IP) and mass spectrometry analysis for the IP products, and identified specific IP products. Double-immunostaining experiments using the neoblast Ab, and the specific Ab against the identified factor, revealed that the staining pattern merged well in Muse cells. Furthermore, we found that some cells in connective tissues of human pancreas were specifically double-stained with the specific Ab and the Muse cell marker SSEA-3 Ab. Collectively, we identified strong candidates of common proteins in multipotent adult stem cells that were conserved from planarian to human.

(COI: No)

P2-008

A molecular mechanism underlying planar cell polarity orientation in Drosophila

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How individual cell polarity becomes aligned along a global axis within a tissue is a central question in developmental biology. In Drosophila, planar cell polarity (PCP) molecules such as Dachsous (Ds) and Four-jointed (Fj) may function as global directional cues orienting cellular asymmetry, which is manifested as polarized localization of PCP core proteins such as Frizzled (Fz). However, the relationship between the Ds/Fj gradients and Fz asymmetry in the eye is opposite to that in the wing, thereby causing controversy about how these two systems are connected. Here, we show that this relationship is determined by the ratio of two Prickle (Pk) isoforms, Pk and Spiny-legs (Sple). Pk and Sple have antagonistic functions and form different complexes with distinct subcellular localizations. In wings where a Pk:Sple ratio representative of the eye was artificially created, Sple-Dachs cooperation polarized Sple at the cell edge exhibiting the highest Ds level, leading to a reversal of PCP orientation. A mathematical model was used to demonstrate that Sple is the key regulator connecting the Ds/Fj gradients and the PCP core proteins. Our model may explain the previously noted discrepancies in terms of the differing relative amounts of Sple in the eye and wing.

(COI: No)

P2-009

Histological findings of the embryonic lung tissue in *Foxc2* knockout mice

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Foxc2 gene is one of known genes for lymphedema-distichiasis syndrome. Moreover this gene is expressed in several embryonic tissues including endothelial and mesenchymal cells of developing cardiovascular system, and several cancer cells. During the investigation of cardiovascular anomalies in *Foxc2* mutants, we found these mutants also showed thicker alveolar septa than wild type fetuses, although heterozygotes could survive after birth. In this study, we characterized the lung tissues to clarify the possible improvement of *Foxc2* gene in the lung development. Mutant fetuses, at embryonic days 15.5-18.5, were obtained by mating between ICR-*Foxc2* heterozygotes (Control: wild type littermates, WT). To characterize tissue morphology, cryo-sections of the lung were made and stained either with hematoxylin-eosin or immunohistochemically. As markers for the lung epithelium differentiation efficacy, pro-SPC and podoplanin were used. CD31 and type4 collagen were used to estimate the lung maturity. Although *Foxc2* mutants, especially in null fetuses, showed cuboidal type1 alveolar cells and narrower lumens than WT, the immunostainings for CD31 or podoplanin in the mutants showed that the distance between vascular endothelial cells and type1 alveolar cells shortened enough compared with those in WT. These data may indicate that *Foxc2* gene can be involved in the maturation process of type1 alveolar cells.

(COI: No)

P2-010

Serotonin transporter during palate formation in mice

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Background and objectives: Palatogenesis is directed by epithelial-mesenchymal interactions and many factors may contribute to the formation of palate. Previous works suggested that serotonin (5-HT) plays an important part in craniofacial development. However, little information exists on the precise role of serotonin transporter (SERT) in palatogenesis. Here, we assessed the localization of SERT in developing mouse palate to determine whether SERT might function during palate formation.
Materials and Methods: Embryos recovered from timed pregnant C57/BL10 mice were used. We evaluated immunoreactivity for SERT during palate formation. We also examined the localization of Ki-67 and cytokeratin (CK) in order to identify the proliferating cells and epithelial cells, respectively.
Results: No reactivity for SERT was observed in the palatal selves oriented vertically at E13.5. The shelves were horizontally at E14.5 and some epithelial cells at the tip of the palatal shelves had SERT labeling. As palate formation progressed, no SERT labeling was observed in the palate at E15.5. CK showed a similar pattern to SERT, whereas Ki-67 was diffusely distributed in the palate mesenchyme. At postnatal day, Ki-67 was seen in the basal layer and CK was observed in the oral epithelium. In contrast, SERT was detected in the basal and middle layer of oral epithelium of the palate.
Conclusions: These findings suggest that SERT contributed to palate formation. SERT may control the differentiation of the palate epithelial cells.

(COI: No)

P2-011

Expression of Osterix in the cleft palate of A/J mouse embryo

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Cleft lip and/or palate, which are caused by aberrant palatal development, are known as one of the most numerous congenital anomalies in human. Palate development begins around embryonic day 13.5 (E13.5) in the mouse. At E14.5, the shelves elevate to a horizontal position above the dorsum of the tongue and the medial edge epithelium of the horizontal palatal shelves contact, adhere and fuse along their midline, forming a midline epithelial seam. At E17.5, the continuous palate formation separates the oral and nasal cavities for breathing and feeding at the same time. The process of palate development is conserved between humans and mice. The A/J mouse strain, in which the incidence of spontaneous cleft lip and palate is 8%-12.1%, has been used as an animal model for human cleft lip and palate. In the present study, we examined expression of osteogenic markers including Osterix and Runx2, and the chondrogenic marker Sox9, during palate development of the two mouse strains, ICR and A/J, using immunohistochemistry. We found that the palate of A/J mice at E16.5 was smaller when compared to ICR, and that the markers' expression in the developing palate was different between ICR and A/J strains and between individuals with and without cleft palate in A/J mice.

(COI: No)

P2-012

Effect of mesenchymal cells in skeletal muscle myoblast cells stratification

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PURPOSE: To investigate proliferation potency of myoblast cells to reveal mechanism of myoblast cells stratification in the creating rabbit oral three-layer lamination sheet inserted mesenchymal cell layer between epithelial cell and myloblast cell sheets.
METHODS: Skeletal myoblast cells and mesenchymal cells were obtained from rabbit oral mucosal tissue using enzymatic digestion. Skeletal myoblast cells were spread onto each of the 6well inserts and cultured with advanced-DMEM with 10% FCS. 3days later, collagen gels or collagen gels containing isolated mesenchymal cells were laminated onto a cultured skeletal myoblast cells. These laminated sheets were labeled with 10mM BrdU for 48 hours before end of the culture. 2 weeks later, we analyzed the samples for immunohistochemistry, reverse transcriptase-polymerase chain reaction (RT-PCR), and DNA cell cycle analysis by fowcytometry.
RESULTS: Myoblast cell sheets stratification was observed by co-cultured with collagen gels or collagen gels containing mesenchymal cells. As the BrdU corporation and cell cycle analysis shows, the proliferation potency fall tendency observed by co-culture with collagen gel, was not observed in co-culture with a mesenchymal cell.
CONCLUSION: Although the still more examination is required about stratification mechanism of skeletal muscle myoblast cells, it is possibility that the mesenchymal cellsinvolved in maintaining the proliferation potency of myoblast cells.

(COI: No)

P2-013

Role of Semaphorin-Rho signaling in ameloblast differentiation

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During tooth development, ameloblasts differentiate into highly polarized matrix-secreting ameloblasts from oral epithelial cells to form enamel. Recently, we reported Rho kinase regulated ameloblasts differentiation through actin polymerization and cell-cell adhesion. However, the up-stream signal cascade is unclear. Previous reports suggested that Semaphorins, originally identified as axon guidance factors, mediated Rho signaling. Thus, in this study, we explored whether semaphorin regulate ameloblasts differentiation through Rho signaling. Immunohistochemistry of mouse incisors showed that Semaphorin 4D (Sema4D) and its receptor Plexin B1 strongly expressed in the polarized secretory ameloblasts, whereas they weakly expressed in inner enamel epithelial cells. Sema4D recombinant protein increased activity of RhoA and actin polymerization in cultured dental epithelial cells. On the other hands, neutralized antibody of Sema4D resulted in morphologic degeneration and reduction of amelogenin expression. A knock down of PlexinB1 inhibited RhoA activation, actin polymerization and amelogenin expression. Furthermore, The polarization of ameloblasts in transgenic mice with ameloblasts-specific expression of RhoA dominant-negative form was hindered. Expression of amelogenin and polymerized actin were also inhibited in the ameloblasts. Together, these results demonstrated that Sema4D-PlexinB1 signal pathway regulated the polarity and matrix production during ameloblasts differentiation through RhoA activity.

(COI: No)

P2-014

Searching the genes regulating myoblast fusion by using perfusion marker

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Myoblast fusion is essential to form the multi-nucleated muscle fibers that provide the contractile strength of skeletal muscle. Myoblast fusion follows an ordered set of events: recognition, adhesion, and plasma membrane union, which results in syncitium formation. However, relatively little is yet known about the molecular mechanism of membrane union. Recently we established a monoclonal antibody reactive to prefusion myocyte that is a fusion-competent, mononucleated muscle cell. By using this antibody, we have created a list of highly expressed genes just before muscle cell fusion. This list includes Jam and Myomaker. It was reported recently that they were critical for myoblast fusion, respectively. However these gene products should associate with other partner molecule to function. We searched another molecule from the list. We found that a zymogen of the digestive enzyme may regulate muscle cell fusion. Besides, listed genes were clustered to several groups by functional annotation. Then we found that the several genes involved in the formation of the tight junction were upregulated in the prefusion myocytes. We will report on the results of analysis of these genes.

(COI: No)

P2-015

5-HT4 receptor-mediated facilitation of neurogenesis of enteric neurons from transplanted brain-derived neural stem cells in the deep tissue of mouse small intestine underwent transection and anastomosis

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Two photon-excited fluorescence microscopy (2PM), can provide deeper optical penetration (several hundred μm) in in vivo preparations. We have used this approach in Thy1-promoter YFP mouse after gut transection and anastomosis. The fetal brain-derived neural stem cell (NSC) transplantation from the tail vein was performed after treatment with red fluorescent cell linker, PKH26. We obtained clear three-dimensional imaging of newborn enteric neurons generated from enteric neural progenitors (enteric NSC; green fluorescence) and those from transplanted NSC derived from the fetal brain (red fluorescence). Number of new neurons from the transplanted NSC was much smaller (approximately 10%) than that from enteric NSC. Neurogenesis was promoted by application of a 5-HT_4-receptor agonist, mosapride citrate (MOS: 100 μM) and this promotion was inhibited by simultaneous application of a 5-HT_4-receptor antagonist, SB-207266 (50 μM). After in vivo imaging, immunohistochemical studies were performed and PGP9.5 positive cells (neurons), and red fluorescence and green fluorescence positive cells were compared by confocal microscope. New enteric neurons overlapped with red fluorescence positive fetal brain-derived NSC and green fluorescence positive enteric NSC in the deep tissue of mouse small intestine.

(COI: No)

P2-016

Ectopic expression of Sema3A in the forebrain impairs the migration of GnRH neurons

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Gonadotropin-releasing hormone (GnRH)-producing neurons that play a role in regulating the reproductive system originate in the olfactory placode and migrate to the hypothalamus. Once GnRH neurons enter the medial forebrain at the site slightly caudal to the olfactory bulb, they undergo axophilic migration in association with a subset of olfactory fibers in a dorsocaudal direction. Class 3 semaphorin (Sema3A) is a secreted protein that functions in repulsive axon guidance and cell migration. We previously showed that chick Sema3A mRNA was expressed in the olfactory bulb and the restricted region of dorsal septum to which GnRH neurons tend to avoid approaching. Most migrating GnRH neurons expressed mRNA of neuropilin-1, a Sema3A receptor. To examine whether Sema3A contributes to the migration of GnRH neurons in the brain, the Sema3A-expression vector was introduced into the medial forebrain of embryonic days 3.5 chick embryos by in ovo electroporation. When misexpression of Sema3A was observed in the rostral part of the medial forebrain 3 days after the treatment, GnRH neurons migrated in a short distance along the medial forebrain surface, but could not proceed to the dorsal septum. In another case, many GnRH neurons were clustered at the entry point of the medial forebrain. These results suggest that Sema3A plays a chemorepulsive role in the migration of GnRH neurons in the brain.

(COI: No)

P2-017

Surrounding cells affect gene expression pattern of human beta-defensins and the shape of population in squamous cell carcinoma cells *in vitro*

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This study aimed to analyze the variation in gene expression levels of human beta-defensins (*hBDs*) in human oral squamous cell carcinoma cells (OSCC) under the co-culture with murine cells. Cell lines of OSCC (HSC-3, HSC-4) were co-cultured with NIH/3T3 or ATDC5 for 1.5 days. The gene expression pattern of the *hBDs* was investigated by a real-time RT-PCR. The expression patterns of *hBDs* of OSCC under co-culture were different from those of OSCC cultured on themselves. The expression of *hBD1* increased significantly when co-cultured with NIH/3T3, however, decreased significantly when co-cultured with ATDC5. Expression of *hBD2* and *hBD4* tended to decrease in co-culture. In a microscopy, small colonies of OSCC surrounded by NIH/3T3 cells were found at 1.5 days. Whereas, no obvious colonization of OSCC was found in the co-culture with ATDC5. Positive signals for anti-HBD1 antibody were found in OSCC aggregations co-cultured with NIH/3T3, however, the weak signals were found in OSCC cells co-cultured with ATDC5. These results suggested that the expression pattern of *hBDs* of OSCC is dependent on the co-culture partner. The different expression of *hBD1* may cause under the different morphology of OSCC population.

(COI: No)

P2-018

Mechanisms and roles of autonomous cell movements in embryonic cells during amphibian gastrulation

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Gastrulation is one of important steps for morphogenesis during multicellular animal embryogenesis. Morphogenetic movements during gastrulation relocate embryonic cells to form three germinal layers (ectoderm, mesoderm and endoderm). Unique autonomous cell movements are known in isolated embryonic cells from amphibian gastrula. However, little is known about their mechanisms. In this study, we investigated the mechanisms and roles of autonomous cell movements in embryonic cells isolated from amphibian gastrula of Japanese newt. Histochemical experiments and live cell imaging using Ca^{2+} cannel activators or inhibitors revealed new findings in relation to embryonic cell movements. Isolated presumptive ectodermal cells carried out mainly circus movement that is based on plasma membrane blebbing. Isolated presumptive mesodermal and endodermal cells, on the other hand, carried out mainly vermiform movement that is based on elongation of cellular body. Their two types of autonomous cell movements in the isolated gastrula cells are regulated by different intracellular Ca^{2+} signaling systems and localized actin polymerization. These findings suggest that development and formation of Ca^{2+} signaling mechanisms depending on a type of germinal layers play an important role in the initiation and execution of morphogenetic cell movements during gastrulation.

(COI: No)

P2-019

Impaired development of left anterior heart field by ectopic retinoic acid causes transposition of the great arteries in the chick embryonic heart

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Background: Transposition of the great arteries (TGA) is one of the most often diagnosed cyanotic congenital heart defects at birth. One of the etiologies causing TGA morphology is the disruption of the left-right axis development. The anterior heart field (AHF) resides in the anterior pharyngeal arches and secondary heart field (SHF) in the coelomic mesoderm dorsal to the heart outflow tract migrate to form right ventricle as well as conotruncus. We previously reported that each heart field contributes to form distinct conotruncal region (Dev Dyn 241:284-293, 2012). The aim of this study is to find the responsible AHF/SHF region, of which abnormal development causes TGA.
Results: We placed a retinoic acid (RA)-soaked bead to the left, right or both sides of AHF of the first and second pharyngeal arches (or SHF) at stage 12 to 14 (embryonic day 2) chick embryos and examined the conotruncal heart defect at stage 34 (ED 8). TGA was diagnosed in embryos, to which RA-soaked bead had been placed on the both sides of AHF or left AHF at stage 12. AHF exposed to RA showed a reduced expression of isl1 and failed to migrate to the conotruncus leading its truncation. In cultured AHF, RA suppressed the expansion and differentiation of cardiomyocytes.
Conclusion: Left AHF in the anterior pharyngeal arches of stage 12 chick embryo is the responsible region of which impediment causes TGA morphology.
(COI: No)

P2-020

Remodeling from hemangioblasts to endocardial cells in the chick embryo

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The development of blood vessels occurs by two different processes: vasculogenesis and angiogenesis. Recent studies have suggested the endocardial cells would arise via the process similar to vasculogenesis during cardio-genesis. However, a detailed morphological analysis on the differentiation of hemangioblasts into endocardial cells has not been confirmed until now. In the present study, we report the morphological process on the differentiation of hemangioblasts into endocardium cells. Chick embryos, 4-4 1/2 days (HH stages; 23-24) were mainly used for this study. Samples were fixed with Zambonin's or Karnovsky's fixative solution for a light microscopy and for eletron microscopic analysis, respectively. Furthermore, immunohistochemistry for FLK-1 was visualized with SAB method. Some of FLK-1 positive cells were observed near the endocardium covering the cardiac lumen. Various differentiated precursor cells from hemangioblasts into endocardial cells were observed at electron-microscopic levels. Some cells had granular reticulum and Glogi-complex associated with large vacuoles containing fine-filamentous substance in their lucid cytoplasms. These cells appeared to be balloon-shaped, adhering on the luminal surface of endocardial cells. Some cells labeled with anti-FLK-1 antibodies might be finally differentiated into endocardial cells. The present results support the hypothesis that endocardium might be constructed via the vasculogenesis during cardio-genesis.
(COI: No)

P2-021

Immunohistochemical observation of the aorta and heart outflow tract during the initial formation of proximal coronary arteries

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Morphological mechanism that establishes the proximal coronary arteries is largely unknown. Using quail embryonic hearts we investigated the wall structure of the aorta and ventricular outlet during the formation of the proximal coronary arteries. Immunohistochemistry showed that tropoelastin (TELN) and fibrillin-2 (FBN2) were already deposited in the distal region of the aorta and inner mesenchyme of the myocardial sleeve prior to the formation of the primitive coronaries at 120-128 hours incubation. Smooth muscle α-actin (SMA) was accumulated in the aortico-pulmonary (AP) septum of the myocardial sleeve. Later at 132-134 hours incubation, primitive coronary arteries began to develop in the right and left coronary sinuses. At this time, SMA positive cells were diminished not only in the AP septum but also in the proximal aorta. Elastic fibers developed in the aortic wall did not extend to the aortic bulb (mesenchymal gap), which were located between the aorta and myocardial sleeve. At 138-152 hours incubation, SMA was accumulated again in the AP septum; and TELN and FBN2 were distributed over the aortic media and the inner mesenchyme of myocardial sleeve. Aortic elastic fibers were extended to the inner mesenchyme of myocardial sleeve. These observations suggest that mesenchymal gap between the aorta and the myocardial sleeve may play a role in the initial formation of the proximal coronary arteries. (This work was supported by Saitama Prefectural University Research Grant.)
(COI: No)

P2-022

Effects of FoxO1 on angiogenesis in the retina

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FoxO1 is a mammalian homolog of Daf-16, known as a longevity gene in C.elegans. FoxO1 is a transcription factor which controls cell cycle arrest, apoptosis, stress resistance, and energy metabolism. Since KO mice die around embryonic day 11 due to impaired angiogenesis, FoxO1 is thought to have an important role in vascular development. However, its mechanism has not completely been clarified. In order to elucidate its function in vascular development, we focused on its role in the endothelial cells during the postnatal angiogenesis in the retina. First we revealed the distinct localization pattern of FoxO1 protein in endothelial tip cell, which exists in the developing vascular front, in the WT mice retina. Secondly we examined which of the upstream signals control the localization by immunohistocmistry with antibodies against phosporylated Akt, ERK and JNK. Furthermore we examined impaired angiogenesis in detail in endothelial-cell specific FoxO1 KO mice. The length of the newly formed vessel was reduced and the branch points and the number of tip cells were increased in FoxO1 KO mice. Finally, to find out genes responsible to the abnormalities, we applied in situ hybridization histochemistry for some genes rich in the tip cells, such as PDGF-B, Ang2 and ESM, to WT and KO mice and identified some candidate genes. Taken together, FoxO1 was suggested to modify angiogenesis through transcriptional regulation of some genes in the endothelial tip cells.
(COI: No)

P2-023

Early life stress reduces BDNF expression and its related factors in rat hippocampus during brain development

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Early life stress interrupts brain development through the disturbance of various neurotransmitter and neurotrophic factor activities, but the details remain unclear. Brain-derived neurotrophic factor (BDNF) is one of the important trophic factors involved in neuronal growth and synaptic connection during early postnatal period. Some previous studies indicated that stress suppresses BDNF-stimulated signal pathway. We examined that how maternal separation (MS) stress influences BDNF expression and its related factors between neonatal and weaning period. The SD rats were individually separated from their dams for 3h twice-daily during postnatal days (PDs) 2-20, and the hippocampus on PDs 7, 10, 14, and 21 were analyzed using real-time RT-PCR and western blot. MS decreased mRNA and protein levels of BDNF on PD7, but did not affect on PD10, 14, and 21. In addition, MS decreased expression and/or activation of BDNF-related factors such as ERK signaling, GABA synthetic enzymes, and cholesterol synthetic enzymes on PD7. Given functional synapses are commenced to form and GABAergic inhibition is recruited around PD7, these alternations potentially disrupt normal balance of brain development.
(COI: No)

P2-024

DINE functions as a protease required for the motor nerve terminal arborization and neuromuscular junction formation

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Damage-induced neuronal endopeptidase (DINE) is a unique membrane-bound metalloprotease that we identified as a nerve injury-inducible gene. DINE-deficient mice (DINE KO) die of respiratory failure immediately after birth, because the phrenic motor nerve fails to arborize and form neuromuscular junction (NMJ) in diaphragm. This suggests that DINE plays a crucial role for motor neuron (MN) development as well as NMJ formation, although the physiological function of DINE remains unclear. Recent reports have shown that some metalloprotease family members function independent of their protease activity. To clarify the significance of DINE protease activity in developing MN, we performed a rescue experiment of DINE KO phenotype by crossing transgenic mice (Tg) overexpressing either wild type (WT) DINE or protease active site-deleted (mut) DINE specifically in MN. The overexpression of WT DINE rescued the abnormal nerve arborization and NMJ formation in DINE KO, while that of mut DINE failed. In addition, more detailed histological analysis of DINE KO revealed that the immature Schwann cells (SCs) along the axons of DINE KO showed abnormal morphology and alignment. Consistent with this finding, the expression of SC differentiation marker Oct6 decreased in DINE KO. SCs co-cultured with DINE-deficient MN were not capable of making ordinary association and alignment to the axons. These findings suggest that axonal DINE has a protease activity which may influence the axon-SCs interaction and thereby form proper nerve arborization and NMJ.
(COI: No)

P2-025

What is the crucial factor that governs the regenerative capacity of the spinal cord after injury in Xenopus?

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In mammals including human, anatomical reconstruction after spinal cord injury (SCI) is limited thus functional recovery hardly occurs. Although so many attempts have been done to develop the treatment for SCI, we have not reached the drastic treatment that enables the functional recovery. To elucidate the really fundamental factor that determines the regeneration capacity after SCI, we utilize Xenopus as the animal for studying the regeneration capacity after SCI. Because Xenopus tadpole is known to have the great capacity of regeneration, in which the spinal cord spontaneously regenerate to achieve the almost complete functional recovery even after complete transection, and to gradually loose its regenerative capacity through the metamorphosis, fascinating us to compare the reaction after SCI between the regenerative and no-regenerative stages. To elucidate the basic capacity of stem/progenitor cells existing in the normal spinal cord of Xenopus tadpole, we analyzed the expression patterns of transcription factors regulating the cell-linage specification in the spinal cord and of cell cycle markers in the regenerative and non-regenerative stage of Xenopus spinal cord with comparison of those observed in the embryonic mouse spinal cord.

(COI: No)

P2-026

Role of neuropeptide PACAP in hematopoiesis via its specific receptor PAC1R

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a multifunctional neuropeptide and contributes to anti-apoptosis, anti-inflammation, cell proliferation and differentiation. However, the role of PACAP in hematopoiesis is still unclear. The purpose of this study is to investigate the localization of PACAP and specific PAC1 receptor (PAC1R), and regulatory action of PACAP in mouse bone marrow (BM). The mRNA and protein expressions of PAC1R were detected in BM aspiration and tissues by RT-PCR and immunohistochemical staining. In particular, PAC1R strong immunoreaction was co-localized with CD34 $^{+}$ hematopoietic stem/progenitor cells (HSPCs). By using flow cytometry (FCM) analysis, % PA1CR was found rich in HSPCs population (CD34 $^{+}$/SCA1 $^{+}$), seemed to decrease with lineage maturation (low % in Gr-1 $^{+}$/CD34 $^{-}$ and CD45R^{+}/CD34^{-}populations). Meanwhile, the colonies-forming unit counts of HSPCs were increased by PACAP. Few PACAP mRNA was detected in the BM aspiration. However, lumbar 1-4 paravertebral ganglions were retrograde traced by flouro-gold from tibial BM. In these ganglions, neuron cell bodies were strongly expressed with tyrosine hydroxylase and PACAP. Therefore, PACAP from nervous system may promote the proliferation of HSPCs via PAC1R signaling pathway.

(COI: No)

P2-027

Transcription factor FoxP1 is involved in the induction of apoptosis specific to the cervical spinal cord of chick embryo

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During the early development of chick embryo, a certain population of motoneuron within the cervical spinal cord undergoes apoptosis between embryonic day 4 and 5. However, the identity of this specific population remains largely unknown. Recently, it has been reported that transcription factor FoxP1 plays a critical role in defining the motoneuron identity in brachial, thoracic, and lumbar spinal cord. However, role of FoxP1 in the cervical region remain unclear. To elucidate the function of FoxP1 regarding apoptosis, we previously examined the effect of overexpression of micro RNA-9 (miR-9), which is known to suppress *FoxP1*, and demonstrated that apoptosis in the cervical spinal cord is reduced by miR-9 overexpression. However, miR-9 also suppresses *Onecut1* (*Oc1*), the expression of which initiates earlier than and overlaps with *FoxP1* expression in the developing spinal cord, and is likely to suppress some other unknown target genes. Thus, the overexpression of miR-9 is an ambiguous condition to examine the precise function of FoxP1. In the present study, we have designed shRNA expression vector that specifically repress *FoxP1*. When the shRNA against *FoxP1* was induced in the cervical spinal cord by electroporation, the number of FoxP1^{+} cells was reduced to approximately 60% compared to the contralateral side. In contrast, the numbers of Oc1^{+} or Lhx3^{+} motoneurons were unchanged. In this condition, signals of TUNEL or active caspase3 were reduced, indicating that FoxP1 is required for the induction of apoptosis in the developing chick cervical spinal cord.

(COI: No)

P2-028

The evolutionary origin of cerebellar neural circuit in vertebrates

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In agnathans including lamprey and hagfish, the cerebellum consists of immature corpus cerebelli and few commissural tracts. By contrast, chondrichthyans, a group of gnathostome, possess the well-organized corpus cerebelli and the elaborated neural connection with the precerebellar nuclei. These observations indicate that the cerebellar neural circuits have been improved after the sprit between agnathans and gnathosotmes. To clarify the origin and evolution of the cerebellum, we investigated the developmental mechanism to form the spinocerebellar tract, which is conserved in every vertebrate.

We injected the neural tracer into the spinal cord to visualize the spinocerebellar tract in various vertebrate embryos, and compared its trajectory with the expression pattern of *Slit2*, a repulsive axon guidance molecule. In both lamprey (*Lethenteron japonicum*) and gnathostomes including mouse and *Xenopus laevis*, the spinal ascending axons crossed the dorsal midline at the posterior side of midbrain-hindbrain boundary, corresponding to the *Slit2*-negative region. Moreover, treatment with *Slit2* antisense morpholino disrupted the spinocerebellar tract in *Xenopus* embryo. These data suggest that *Slit2*-dependent axon guidance mechanism, which is conserved through the vertebrate evolution, plays an important role for the formation of spinocerebellar tract.

(COI: No)

P2-029

Specification of select hypothalamic circuits and innate behaviors by the embryonic patterning gene, Dbx

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The hypothalamus integrates information required for the output of a variety of innate behaviors such as feeding, mating, aggression and predator avoidance. Despite an extensive knowledge of hypothalamic function, how embryonic developmental programs specify circuits that regulate innate behaviors remains unknown. Here, we find that in the hypothalamus the developmentally regulated homeodomain-containing transcription factor, Dbx1, is selectively required for the generation of subclasses of neurons within the feeding-associated lateral hypothalamic area/zona incerta and arcuate nucleus. Consistent with this specific developmental role, Dbx1 hypothalamic-specific conditional knockout mice display alterations in energy homeostasis and innate stress responses to predator odor, but not other innate behaviors such as mating or conspecific aggression. Thus, Dbx1 is a common developmental genetic mechanism for specification of neurons in two distinct but functionally related hypothalamic nuclei, and links energy homeostasis and innate stress.

(COI: No)

P2-030

The mechanism for induction of ascidian peripheral neurons

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The vertebrate peripheral nervous system (PNS) originates from the neural crest and neurogenic placodes, which arise at the boundary between the neural plate and non-neural ectoderm. The neural crest and neurogenic placodes are considered to be vertebrate innovations, but their evolutionary origins remain unresolved. To gain more insight into the evolution of the PNS, we investigated the development of ascidian PNS, because ascidians are protochordates closely related to vertebrates and expected to provide information about the transition between invertebrates and vertebrates. We found that epidermal sensory neurons (ESNs), which mainly constitute the entire peripheral nervous system of the ascidian young tadpoles, are derived from the neural plate border, as is the case in the vertebrate PNS, and demonstrated that FGF, Nodal and BMP signaling are required for ESN specification. Gene knockdown experiments showed that moderate levels of BMP activity induce ESNs at the tailbud stage, suggesting that the role of BMP signaling in PNS formation is conserved among chordates. We also found that Nodal signaling regulates expression of BMP signaling molecules in the lateral neural plate, and consequently specifies ESNs, which clearly differs from the BMP gradient model proposed for vertebrate neural induction.

(COI: No)

P2-031

The role of BMP in the process of the developing dentate gyrus

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In the process of dentate gyrus (DG) formation, neural stem cells (NSCs) and progenitors in near the cortical hem proliferate and migrate to form granule cell (GC) layer. Our previous analysis using transgenic mice expressing GFP under the control of glial fibrillary acidic protein (Gfap) promoter (Gfap-GFP mice) reveals that during embryonic stages GFP-expressing cells confined to the dentate primordium produce granule cells (GCs). Since DG formation is reported to be impaired in the BMP receptor (BMPR) deficient mice, we thought that the malformation is due to the reduction of Gfap-expressing cells by deficient of BMP signaling. Here we investigated the role of BMP signaling in the production of GCs by Gfap-expressing cells. When neurosphere assay was performed using embryonic hippocampal cells from Gfap-GFP mice, Gfap-expressing neurospheres are detected, suggesting that they contain NSCs. RT-PCR analysis revealed that bone morphogenetic proteins (BMP2 and BMP4) are strongly expressed by the developing hippocampus. Thus, to examine the effect of BMP signaling in vivo, Noggin, BMP signaling inhibitor or dominant negative form of BMPR was introduced in the embryonic brain. The suppression of BMP signaling reduced Gfap-expressing cells in the developing hippocampus as well as Prox1$^+$ GCs. Taken together, these results indicate that Gfap-expressing NSCs that contribute to the formation of the DG are induced by BMPs that are abundantly contained in the developing hippocampus.

(COI: No)

P2-032

Cell-tracing Analysis for Progenitor Cell Migration in the Embryonic Dentate Gyrus

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In general, neurogenesis occurs during embryonic and early postnatal stages, and ceases at adult stage. However, the dentate gyrus (DG) continues neurogenesis from embryonic to adult stages. In the adult DG, granule neurons are generated in the subgranular zone, while during embryonic period, dentate neural progenitors are initially produced in the ventricular zone (VZ), and then migrate through the suprafimbrial region to the subpial region (SP) where a new proliferative zone is formed to develop the presumptive dentate gyrus. During the migration, the progenitors differentiate into granule neurons or maintain property of neural progenitors that further contribute to perinatal and postnatal neurogenesis. Although the migration of the neural precursors and relocation of the region of neurogenesis are key processes for the formation of the DG, the exact temporal and spatial patterns are still unknown. To address the problem, we performed cell-tracing analysis of the DG by in utero electroporation. RFP-positive cells originated from the VZ migrated to the DG. Immunohistochemical studies revealed that the RFP+/Tbr2+ cells were present in the SP, whereas the RFP+/Sox2+ cells were localized in both the SP and the hilus. Moreover, we performed time-lapse imaging in cultured hippocampal slices and found some types of cell migration in the DG: pia-touching cells, presumptive hippocampal fissure-touching and somal translocation-like cells. We will discuss possibility that the correlation between cell-type specification and modes of cell migration.

(COI: No)

P2-033

Tangential cell migration in the superficial layers of the developing optic tectum

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The optic tectum is composed of multiple layers, which are formed by the radial and tangential migration during development. We have previously reported a population of tangentially migrating cells in the deep layers of the developing chick optic tectum. Here, we report another tangential cell migration in the superficial layers. When the ventricular cells of chick tectum were labeled by the electroporation of GFP-expression vector at E4.5-E5.5, GFP-labeled cells migrated radially and some of them turned horizontally in the superficial layers after E7.0 and began to spread throughout the tectum. In contrast to the tangential migration in the deep layers, which is strictly guided by the tectal efferent axons in dorso-ventral and ventro-dorsal directions, these tangential migrants in the superficial layers moved freely in multiple directions with bifurcated leading processes. We are currently trying to identify their cell fates after the migration to characterize this novel superficial migration in the developing optic tectum.

(COI: No)

P2-034

Expression and function of ADP-ribosylation factor 6 (Arf6) in the neuronal migration during cortical layer formation

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Cortical layer formation in the cerebral cortex is one of the typical events in the mammalian brain. Neurons that are born in the ventricular zone migrate to the pial surface with an inside-out manner along the fiber of radial glia. Recent studies revealed that transmembrane proteins such as connexins, integrins, and N-cadherin, regulate neuronal migration through cell-cell and/or cell-matrix interactions, and their expression on the plasma membrane is tightly regulated by vesicle trafficking factors that are involved in the process of secretion, endocytosis and recycling. However, it largely remains unclear the mechanistic details of how vesicle trafficking factors regulate neuronal migration. In this study, we examined the functional role of ADP-ribosylation factor 6 (Arf6), a critical regulator of endosomal trafficking, in the cortical layer formation. In situ hybridization analysis revealed that Arf6 mRNA was expressed in all layers including ventricular zone, intermediate zone, and cortical plate in the dorsal pallium of embryonic cerebral cortex. Knockdown of Arf6 by in utero electroporation resulted in the decrease in the cell population invading to layer II-IV. Furthermore, time-lapse observation demonstrated that neuronal migration was delayed in the intermediate zone by knockdown of Arf6. These results suggest that Arf6 regulates the neuronal migration in multipolar mode through vesicle trafficking.

(COI: No)

P2-035

Cell divisions of neural progenitor cells are regulated by NRG1-ErbB signaling in the developing zebrafish optic tectum

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Post-mitotic neurons are generated from neural progenitor cells (NPCs) at the expense of their proliferation. Molecular and cellular mechanisms that regulate neuron production should impact on the size and shape of the brain. While transcription factors govern progression of neurogenesis as cell-intrinsic mechanisms, recent studies show regulatory roles of several cell-extrinsic signaling molecules in production of NPCs from neural stem/radial glial cells. However, it remains elusive what regulates production of post-mitotic neurons from NPCs. In the optic tectum (OT) of zebrafish embryos, newborn neurons accumulate in the basal-to-apical direction. Here, we show that this neurogenesis depends on Neuregulin 1 (NRG1)-ErbB signaling. Transient treatment with an ErbB inhibitor, AG1478 impairs mitoses in the sub-basal region of the OT prominently. Removal of AG1478 resumes sub-basal mitoses and basal-to-apical accumulation of neurons without affecting mitoses in the apical ventricular (V) region, suggesting critical roles of ErbB signaling in mitoses of post-mitotic neuron production. Depletion of NRG1 type II isoform (NRG1-II) impairs both mitoses in the sub-basal and apical regions. Injection of soluble human NRG1 into the developing brain ameliorates neurogenesis of NRG1-II-depleted embryos. These results imply that NRG1-ErbB signaling promotes neurogenic competence of NPCs in the developing vertebrate brain.

(COI: No)

P2-036

Newborn mice exposed prenatally to bisphenol A show hyperactivity and defective neocortical development

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The central nervous system is especially sensitive to toxic insults during development. Prenatal administration of bisphenol A (BPA) induces histologic changes in the dorsal telencephalon of the embryo. Whether these changes affect the morphogenesis and maturation of neuronal function of the newborn neocortex, however, is unknown. To evaluate the neurodevelopmental and behavioral effects of prenatal BPA exposure at 20 and 200 μg/kg/day in newborn mice, we performed a detailed histologic analysis of the neocortex and tested for the presence of behavioral abnormalities in newborn mice prenatally exposed to BPA using the behavioral test. Observations of newborn mice prenatally exposed to BPA revealed abnormal neuronal distribution and layer formation, hypoplasia of layer 6b, and abnormal dopaminergic neuronal projections in the neocortex. Further, the newborn mice exhibited hyperactivity. These findings suggest that prenatal BPA exposure induces neurobehavioral toxicity associated with abnormal dopaminergic neuronal projections, and abnormal corticogenesis. Histologic and behavioral analyses of newborn mice are considered useful for assessing the neurodevelopmental and behavioral toxicity of chemicals.

(COI: No)

P2-037

Live-cell imaging of nephrogenesis

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The mammalian kidney arises via reciprocal interactions between ureteric bud (UB) and the surrounding metanephric mesencyme (MM). UB grows and branches repeatedly in the MM. Around the UB tip, MM cells become densely packed to form a cap condensation (CC). A subset of CC cells then forms a pretubular aggregate (PA). As a result of mesenchymal epithelial transition, PA forms a single epithelial renal vesicle, which is a progenitor cell population for the all nephron epithelia. These processes of kidney development have been mostly studied by histology of the fixed kidney rudiments of various developmental stages. Thus, there are considerable limitations in understanding the dynamics of cellular events during nephrogenesis. Here, we cultured the rudimental metanephros of embryonic day-11 mice in a medium containing a non cell-permeable fluorescent tracer, and observed cell behavior by the time-lapse confocal microscopy. This method allowed us to trace the migration and proliferation of individual UB or MM cells, as a shadowgraph movie. We found extensive cell divisions in the CC and PA, and some in the UB. These were confirmed by the EdU (a thymidine analogue) incorporation assay. Roles and fates of these highly proliferative cells on the nephrogenesis will be discussed.

(COI: No)

P2-038

FGF9 and BMP4 regulate the competence of Wolffian duct

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The metanephros starts to develop by ureteric bud formation from Wolffian duct (WD) in response to GDNF secreted from metanephric mesenchyme. BMP4 secreted by surrounding mesenchyme has been reported to suppress ureteric bud formation, thereby contribute to the orthotopic formation of ureteric bud. However, the mechanisms of this suppression have not been fully elucidated. We have reported that FGF9 expressed in both WD and its mesenchyme supports the survival and responsiveness of WD, maintaining the gene expression of GDNF receptors Ret and Gfra1, Fgf9 and Sox9. In the present study, we assessed the effect of FGF9 on the expression of growth factors including BMP4 in the mesenchyme, and also examined the effect of BMP4 on WD in vitro. In rat embryos at E12 and 13, expression of several growth factors such as Bmp4, Wnt4 and Wnt2b was verified in the WD mesenchyme. Addition of FGF9 to the culture of mesenchyme significantly increased the expression of CyclinD1, while decreased that of Bmp4, Wnt4 and Wnt2b. Addition of BMP4 to the FGF9-maintained WD culture significantly decreased the expression of Ret, CyclinD1 and especially Fgf9, whereas WNT4 showed no apparent effect on these gene expressions. From these findings, FGF9 of WD and mesenchymal BMP4 appeared to reciprocally inhibit at the gene expression level. FGF9 may enhance competence of WD by suppressing mesenchymal Bmp4 expression. In turn, mesenchymal BMP4 may suppress ureteric bud formation, at least in part, through downregulation of Ret and FGF9 in WD.

(COI: No)

P2-039

Interkinetic nuclear migration in the mouse embryonic ureteric epithelium

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Purpose: Interkinetic nuclear migration (INM) is the phenomenon that progenitor cell nuclei migrate along the apicobasal axis of the pseudostratified epithelial layer synchronously with the progression of the cell cycle, and is suggested as a regulatory mechanism of stem cell proliferation. INM has been reported in epithelia of ectodermal origin. We previously reported INM in the endoderm-derived midgut epithelium in mice. We here examined whether INM exists in the mesoderm-derived ureteric epithelium.
Methods: At E11.5, E12.5 and E13.5, C57BL/6J mouse dams were injected with bromodeoxyuridine (BrdU) and sacrificed 1, 2, 4, 6, 8, 10 and 12 hours later to collect embryos. Transverse sections were BrdU-immunostained. We measured the position of BrdU-positive nuclei in ureteric epithelia along the apicobasal axis at each time point. We analyzed the distribution patterns of BrdU-positive nuclei in histograms at each point using the multidimensional scaling method.
Results: Changes in nucleus distribution patterns that suggest nucleus movement characteristic of INM was found in ureteric epithelia. Nucleus distribution patterns varied depending on the date.
Conclusions: INM exists in the ureteric epithelium of mesoderm origin.

(COI: No)

P2-040

Cathepsin D-deficient mice exhibit impairment of postnatal growth of kidney and liver cells

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Cathepsin D (CTSD) is the principal lysosomal aspartate protease and expressed in most of tissues, but the level of expression varies considerably. Mice deficient in CTSD, generated by gene targeting, develop normally during the first 2 weeks, stop thriving in the third week and die in a state of anorexia at day 26 ± 1. An atrophy of the ileal mucosa first observed in the third week progresses towards widespread intestinal necroses accompanied by thromboemboli. From these results, Saftig et al. (1995) suggested that vital functions of cathepsin D are exerted by limited proteolysis of proteins regulating cell growth and/or tissue homeostasis. The mechanism of growth disorder caused by CTSD deficiency is still unknown. To study the relationship between CTSD and cell proliferation, immunostaining for ki67, a cellular marker for proliferation, was performed in the kidney and liver of CTSD-KO mice. Positive staining for ki67 was significantly less in number in proximal renal tubular cells and hepatocytes of CTSD-KO mice at the age of 23-25 days (p23-p25) after birth than in those of wild-type littermates. These results suggest that proliferation and/or cell cycle were impaired in comparison with age. As weight difference between the CTSD-deficient and wild-type mice became remarkable after p14, CTSD may be involved in the activation of some kinds of growth factors for the small intestine, liver and kidney.

(COI: No)

P2-041

Prosaposin mRNA and protein expression in rat testis

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Prosaposin is the precursor of sphingolipid hydrolase activator proteins called saposins (saposin A-D). However, prosaposin is not merely a precursor of saposins, it also functions as a trophic factor. Prosaposin is found in cerebrospinal fluid, bile, pancreatic juice, milk, and semen. In this study, we performed immunohistochemical and in situ hybridization analyses to clarify the role of prosaposin during spermatogenesis in rat testis. We performed triple immunostaining using specific anti-prosaposin antibodies. Intense prosaposin immunoreactivity was observed mainly in spermatogonia, spermatocytes, spermatids, and Sertoli cells. We also examined the expression patterns of alternatively spliced forms of PSAP mRNA in rat testis. In rats, alternative splicing of the PSAP gene generates two forms of mRNA: Pro+9, containing a nine-base insertion, and Pro+0, which lacks the insertion. According to Madar-Shapiro et al. (1999), the Pro+9 form is preferentially secreted from cells, whereas Pro+0 is mainly found in lysosomes. In the present study, prosaposin mRNA was expressed in the basal half of the testicular tubules, while the secreted form was mainly expressed in testis.

(COI: No)

P2-042

Roles of Bmp signaling in the L-R asymmetric development of female chicken gonads

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The primordial gonads are formed on the left and right ventromedial surfaces of the mesonephros. In the chicken embryos, as in many other avian species, only the left ovary develops while the right one eventually degenerates after sexual differentiation. To understand molecular mechanisms underlying this remarkable phenomenon, we investigated the roles of *Bmp* in the L-R asymmetric development of female chicken gonads. We observed expression pattern of *Bmp7* and *Smads* and overexpressed *Follistatin* (*Fst*), an antagonist of *Bmp*, in the presumptive gonadal region and analyzed gene expression patterns in both ovaries. The overexpression of *Fst* caused the right ovary to form cortex including germ cells. In addition overexpression of *Fst* induced *Pitx2* expression in the right gonad and stimulated proliferation of somatic cells and germ cells. These results strongly suggest that *Bmp* signaling may play an important role in the L-R asymmetric gonadogenesis in female chicken embryo.

(COI: No)

P2-043

Decidual natural killer cells uptake placenta-associated miRNAs during early pregnancy

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Objective: Decidual natural killer (dNK) cells play important roles in the maintenance of early pregnancy. We hypothesized that circulating placenta-associated miRNAs might be transferred via exosomes from placental trophoblasts into maternal immune cells. We investigated whether dNK cells contain the chromosome 19 miRNA cluster (C19MC) miRNAs, which are expressed exclusively in the placenta.
Methods: Decidual tissue and peripheral blood samples from patients who gave informed consent were aseptically obtained after legal abortions (at 6-7 weeks of gestation, n = 3). The expression levels of miRNAs were examined by real-time PCR using a TaqMan microRNA Assay, and gene expression profiling was conducted using Agilent microarrays. Integrated miRNA-mRNA expression profiling and pathway analysis were performed using Ingenuity Pathway Analysis software.
Results & Conclusion: The miRNA array analysis showed that C19MC miRNAs were detected in dNK cells. By in silico analysis, twenty-one C19MC miRNAs targeted many genes that were downregulated in dNK cells compared to peripheral blood NK cells. The miRNA-mRNA network analysis indicates the inhibition of NK cell cytotoxicity by C19MC miRNAs in dNK cells. C19MC miRNAs in dNK cells may contribute to the maintenance of early pregnancy.
(COI: No)

P2-044

The expression of H19 non-coding RNA in developmental stages of the mouse placenta

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Objective: The maternally imprinted gene H19 encodes a non-coding RNA (ncRNA). H19 is strongly expressed during embryogenesis. However, there is little information available on the expression pattern of this gene in placenta development. We examined the expression level of H19 ncRNA in the mouse placenta by real-time quantitative reverse transcription PCR (real-time PCR) and in situ hybridization.
Methods: We studied the expression level of H19 ncRNA in B6D2F1 mouse placentas at four different developmental stages; E7.5, E10.5, E13.5, and E16.5. We extracted total RNA from these different stages of mouse placentas and adult mouse organs (i.e., brain, heart, lung, liver, kidney, intestine, spleen, uterus, ovary, and testis,) and performed real-time PCR for detection of expression of H19 ncRNA. In addition, we investigated expression level of H19 using in situ hybridization using DIG-labelled RNA probe.
Results: We found that H19 ncRNA was exclusively expressed in the mouse placenta by real-time PCR. The expression levels of H19 were higher than those of adult organs examined in this study; the placenta had 80, 800, 1000, and 700 fold higher expression of H19 in E7.5, E10.5, E13.5, and E16.5, respectively when compared to the adult organs. H19 ncRNA was detectable in the placenta using in situ hybridization.
Conclusion: Our findings showed that the placenta expressed H19 ncRNA in a development-dependent manner, especially in the middle and late stages.
(COI: No)

P2-045

Comparative developmental analysis of middle ear formation in mouse and chicken embryos

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How the amniote middle ear evolved remains an intriguing question. Although paleontological studies suggest that the middle ear evolved independently in mammals and diapsids (modern reptiles and birds), little is known about the developmental basis for independent evolution. We have previously found that the relative positions of the primary jaw joint (PJJ: the articulation between the quadrate- and articular-homologue) and first pharyngeal pouch (PP1) led to the coupling of tympanic membrane formation with the lower jaw in mammals, but with the upper jaw in diapsids. In this study, we further compared middle ear formation in mouse and chicken embryos. We found no difference in the expression pattern of genes central to lower jaw specification at comparable stages (E9.5 in the mouse and HH18 in the chicken). However, within a day, these genes were detected more dorsally in the mouse compared to the chicken, resulting in different positioning of the PJJ relative to PP1 in these animals. We also found that the chicken external auditory meatus originates from the ectoderm in the second pharyngeal arch (PA2), not in the first pharyngeal arch (PA1) as in the mouse. These results suggest that although early patterning of the pharyngeal arches is comparable, middle ear formation basically associates with PA1 in the mouse, but with PA2 in the chicken, supporting the idea of independent origin of the middle ear in mammals and diapsids.
(COI: No)

P2-046

Tlx3 promotes glutamatergic neuronal differentiation through the interaction with epigenetic co-factor CBP

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The homeodomain transcription factor T cell leukemia 3 (Tlx3) functions as a selector gene determining glutamatergic cell fate. However, how Tlx3 promotes glutamatergic neuronal specification is unknown. In this study, we show that Tlx3 directly interacts with the epigenetic co-regulator CREB-binding protein (CBP) via its homeodomain. In addition, the interaction between Tlx3 and CBP is enhanced by Pbx3, a member of the TALE family of transcription factors. Using mouse embryonic stem (ES) cells stably expressing Tlx3 to evaluate glutamatergic lineage commitment, we further demonstrate that Tlx3 binds CBP only after neural induction. The expression of Pbx3 was increased upon neural differentiation of ES cells. A deletion mutation in the homeodomain of Tlx3 abolishes glutamatergic neuronal specification of ES cells but has no effect on neural differentiation. Taken together, these data suggest that functional interplay between Tlx3 and CBP plays an essential role in glutamatergic neuronal subtype specification.
(COI: No)

P2-047

Programmed cell death in the developing optic cup in anophthalmia mutant (kAP)-rat

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Introduction: The kAP-rat has anophthalmia, but not macroscopic complications in other organs, unlike other anophthalmia model animals. In our previous study, we showed that the optic cup disappeared between embryonic day (E) 13.5 and E14.5. In this study, we examined programming cell death in the optic cup to analyze the cause of anophthalmia.
Methods: The number of the TUNEL-positive nuclei was counted in the inner and outer layersof the optic cup in female kAP- and control Wistar rat embryos (E12.5). The number of them per unit length was compared between kAP and control embryos.
Result: TUNEL-positive nuclei per unit length were significantly larger in the outer layer of the optic cup in kAP-rat embryos than that in controls ($P = 0.019$ and $P = 0.021$ in the left and right eyes, respectively), whereas there was no significant difference in the number of the TUNEL positive nuclei in the inner layer between kAP and control embryos.
Conclusion: This result suggests that degeneration of the outer layer triggers disappearance of the eye in the kAP-rat.
(COI: No)

P2-048

Morphological analysis of prosensory epithelium in the extension of organ of Corti

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In the development of the mouse cochlea, prosensory epithelium extends along the cochlea spiral. Since the extension requires PCP signal components and non-muscle myosin, and exhibits rosette formation of the epithelial cells, it is thought to be convergent extension. However, detailed mechanisms for the extension remain obscure, since myosin has been reported to accumulate at the cell-cell boundaries parallel to the tissue elongation in the cochlea, which is orthogonal to known accumulating pattern of myosin in a typical convergent extension. We thoroughly analyzed the cellular morphology and distribution of non-muscle myosin proteins in the prosensory epithelium to dissect the processes of cochlear extension. The analyses showed that (1) non-muscle myosin accumulated in punctate dots along the apical cell-cell boundary of each epithelial cell and (2) expression level of non-muscle myosin was dependent on cell types and stages of differentiation. In addition, non-muscle myosin seemed not yet to have accumulated in parallel to the tissue elongation when the rosette formation occurs. The present survey items could be used as indices to evaluate the achievement of each process during the extension and we are intending to evaluate a defect in cochlear extension in Dlg1 gene-targeted mice, which exhibit multiple developmental abnormalities similar to PCP phenotypes. The evaluation is currently in progress and results will be presented on posters.
(COI: No)

P2-049

Possible involvement of olfactory placode-derived neurons in development of the telencephalon

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Olfactory placode (OP), anlage of the olfactory epithelium, is known to produce various kinds of cells in addition to olfactory receptor neurons. OP-epithelial cell migration of chick embryos starts at embryonic day 2.5 (E2.5). The migratory cells are immunoreactive (-ir) for polysialylated NCAM (PSA-NCAM, a marker of immature neurons) and HuC/D (a neuronal marker). The neurons then form a cellular cord toward rostral telencephalon (TEL) and the olfactory nerve axons grow along the cord from E3.5. In the early stage of migration, many neurons migrate towards various directions from the OP or the cellular cord. To know the fate of such migratory neurons, OP-epithelial cells were labeled with GFP or Tol2-GFP vector by electroporation at E2.5 (HH stage 14-18). Embryos were fixed 38 to 72 hours after the treatment and whole-mount specimens were immunostained for PSA-NCAM, and HuC/D or laminin. GFP-labeled cells with HuC/D-ir cell bodies and PSA-NCAM-ir long horizontal processes were detected in the olfactory nerve and in the lateral and medial wall of dorsal TEL of HH stage 21 to 27 embryos. At TEL most GFP-labeled cells were detected on or just beneath the pia mater. In the subpial layer of the dorsal TEL PSA-NCAM-ir neuronal network, which may correspond to Cajar-Retzius cells in mammals were present in E4 to E5 embryos. OP-derived PSA-NCAM-expressing neurons appeared to join the network at later stages. These results indicate involvement of the OP-derived neurons in development of the telencephalon.

(COI: No)

P2-050

Expression of epigenetic factors in the developing mouse retina

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Many studies suggest that epigenetic regulation affects proliferation and cell differentiation during development. Epigenetic modification, such as histone methylation, induce gene activation or silencing by regulating the accessibility of regulatory molecules to chromatin. To understand the role of epigenetic factors in retinal development, we investigated temporal gene expression patterns of histone demethylases and methylases using in situ hybridization. We found that histone demethylases KDM1A, B, KDM2A, B, KDM3A, B, KDM4A, B, C, D, KDM5A, B, C, D, KDM6B were expressed in retinal progenitor cells in embryonic stages. Histone methylases G9a, SETDB1, SUV39H1 also expressed in retinal progenitor cells. These results indicate that many epigenetic factors may regulate the function of retinal progenitor cells during the early retinal development.

(COI: No)

P2-051

Identification of the causal gene of gastrointestinal atresia using medaka mutant

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Gastrointestinal Atresia (GA) is a common feature of congenital malformations and occurs in approximately 1:500 to 1:4000 newborns. GAs can occur as sporadic and are generally thought to originate from mechanical or vascular incidents. However, recent data have showed that genetic components might be also present. To understand the genetic basis of GA, we analyzed medaka mutant which revealed a GA during the embryonic development.

The medaka *g1-4* mutant was isolated in our ENU-driven screen for mutants with defects in embryonic development and organogenesis. *g1-4* revealed GA at 4 to 5 days after fertilization. The carrier pairs had produced around 10% mutant siblings, suggesting that g1-4 was likely a recessive mutant although its penetrance was lower than expected Mendelian frequency.

To determine the molecular structure of the *g1-4* locus, we first mapped it to linkage group 23 (LG 23) using M-marker analysis (Kimura et al., Mech Dev, vol 121 pp915-32, 2004). Subsequently, high-resolution linkage analysis was performed using an F2 mapping panel. This confined the mutation between two markers, *g1-4*_LG23-4.4 and *g1-4*_LG23-4.6, spanning a region of 24 kb. This region contained only one gene. Comparison of genomic sequences between the wild-type and *g1-4* mutant revealed a C to A transversion that led to a premature stop codon in one exon in the mutant.

(COI: No)

P2-052

Evolution of the pectoral fin/limb and the vertebrate neck

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Fin/limb in vertebrates is one of the evolutionary novelties, and its evolutionary origin remains as an enigma. To gain some insight into the question, cell lineage of the lateral plate mesoderm (LPM) was analyzed by using chicken-quail chimera. The most rostral LPM formed pharyngeal mesoderm during the middle stage of the development, and developed cucullaris muscle in the later stage, implying that the region would be head mesoderm. The LPM just caudal to the source of the cucullaris muscle developed the clavicle. Thus, the clavicle adjoins the cucullaris from the early stage in chick. Because the shoulder girdle of the teleosts marks the caudal rim of gill (pharyngeal) arches, the results suggest that, the shoulder girdle is always attached to the head independently of the number of the cervical vertebrae. The infrahyoid muscle in medaka developed adjacent to the fin muscle. These muscles developed from the same somite, and shared the same developmental mechanisms. The mechanism is also used for the development of the infrahyoid muscle in agnathans, lamprey, which do not possess paired fin. Thus, pectoral fin/limb inevitably attaches to the head, implying that it appears to have evolved adjacent to the gill arches, and pectoral fin/limb muscle would have established through co-option of the developmental program of the infrahyoid muscle.

(COI: No)

P2-053

Morphogenesis of the rat glenohumeral joint

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The mechanism of development of the shoulder joint was shown in some studies. The shoulder joint has an interesting structure, for example, SLAP (superior labrum anterior and posterior) and enthesis of rotator cuff. To observe in detail the development of the structure of the shoulder in rat, white Wistar rat embryos of E18 to P1 (postnatal day one) were employed. After mating, the morning when sperm was observed in a vaginal smear was designated as gestational day 0. All the mother rats were anesthetized with ether gas for sacrifice. For paraffin histology, samples of the whole shoulder joint were fixed in 4% paraformaldehyde at 4 oC overnight. Then they were dehydrated in graded ethanol and embedded in paraffin wax. Sagittal and axillar sections were serially cut at 4-6-μm thickness, and were stained with hematoxylin eosin staining. Immunochemical stain of the collagen type I and III was also performed in this study. The shape of rat scapula was more rectangular shape than human that. And acromion is placed on the scapula. In the stage of E18.5, cavitation was clearly recognized in the lateral side of the joint although that was not shown around the articular surface. SLAP and enthesis of the rotator cuff was detected in this stage. In E19.5, vascular formation was recognized in the medial side of the enthesis of the rotator cuff. We showed re-evaluation of the development in the rat shoulder joint.

(COI: No)

P2-054

Generation of Rat-Induced Pluripotent Stem Cells Using Mesenchymal Stromal Cells from a New Model of Metabolic Syndrome

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We recently characterized DahlS. Z-*Lepr^{fa}/Lepr^{fa}* (DS/obese) rats, derived from a cross between Dahl salt-sensitive rats and Zucker rats, as a new animal model of metabolic syndrome (MetS). Although the phenotype of DS/obese rats is similar to that of humans with MetS, the pathophysiological and metabolic characteristics in each cell type remain to be clarified. Hence, the establishment of induced pluripotent stem cells (iPSCs) derived from MetS rats is essential for investigations of MetS *in vitro*. Reports of rat iPSCs (riPSCs), however, are few because of the difficulty. Recently, the advantage of using mesenchymal stromal cells (MSCs) as a cell source for generating iPSCs was described. We aimed to establish riPSCs from MSCs of both DS/obese rats and their lean littermates, DahlS. Z-*Lepr^{+}/Lepr^{+}* (DS/lean) rats. The established colonies showed ES cell (ESCs)-like properties, and the differentiation potential into cells from all three germ layers both *in vitro* and *in vivo*(teratomas). Both riPSCs became adipocytes after induction of adipogenesis. Real-time PCR analysis also revealed that both riPSCs and the adipose tissue from DS/obese and DS/lean rats possess similar expression patterns of adipocyte differentiation-related genes. We succeeded in generating riPSCs effectively from MSCs of both DS/obese and DS/lean rats. These riPSCs may well serve as highly effective tools for the investigation of MetS pathophysiology *in vitro*.

(COI: No)

P2-055

Lumbar lateral plate cells stop migration of thoracic somite cells: an in vitro model of region specific morphogenesis of the chick axial skeleton

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The rib is restricted to the thoracic region of birds and mammals. Although the thoracic somites have potency to form ribs, we have shown that morphogenesis of the rib is depending on circumstance of the somite. When somatic mesoderm of the limb forming region was transplanted into the thoracic region (Liem and Aoyama, 2009) or thoracic segmental plate to lumbosacral region, the grafted mesoderm gave rise to ectopic ribs, which were much shorter than usual (Matsutani et al., JAA cong., 2014). In the latter case, the lateral somite lips derived from the graft did not penetrate the lumbar lateral plate. The lateral plate appears to stop the migration of the somite cells. To examine the interaction between these embryonic tissues, we co-culture the thoracic somites with lateral plate, and found that when the somite cells collided with lumbar somatic mesoderm cells, they rapidly moved away from lumbar somatic mesoderm cells (Matsutani et al., JAA cong., 2014). For quantitative analysis we cultivated the somite and the lateral plate on the 10 μm wide hydrophilic straight paths. When they collided, while thoracic somatic mesodermal cells generated a new protrusion and began to migrate in the opposite direction to the somite cells and, lumbar somatic mesodermal cells remained at the encounter point and inhibited somitic cells migration. The authors declare no conflict interests.

(COI: No)

P2-056

Effect of transplantation of choroid plexus epithelial cells for spinal cord injury in rats

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Although spinal cord injuries have been extensively studied, no effective treatment for spinal cord injury (SCI) is currently available. The development of effective treatments is urgently needed. Recently, transplantation of several kinds of cells for regenerative medicine has attracted a great deal of attention. We previously reported the effect of bone marrow stromal cells (BMSCs) transplantation for SCI, and choroid plexus epithelial cells (CPECs) transplantation for acute ischemic brain injury. CPECs, producing the cerebrospinal fluid, are known to express various neurotrophic factors such as IGFs, FGFs, and EGF. We have detected that cultured CPECs express several neurotrophic factors such as NGF, VEGF, HGF, BDNF, and FGFs. In the present study, we examined the therapeutic effect of CPEC transplantation for SCI in rat. Locomotory behaviors assessed by the BBB score were significantly improved in the cell transplantation group. The transplanted CPECs survived in the spinal cord at least 2 weeks after transplantation, in our previous studies, BMSCs disappeared at 1-2 weeks after transplantation. The differentiation of transplanted CPECs into other neuronal cells has not been identified. Therefore, it is probable that neurotrophic factors secreted from transplanted CPECs might contribute to neuronal regeneration in injured spinal cord. Further studies are needed to explore the therapeutic mechanisms of CPEC transplantation.

(COI: No)

P2-057

Effect of treadmill exercise on the motor recovery and neurogenesis after photochemically induced infarction of unilateral motor cortex in rats

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It is well known that the exercise therapy in rehabilitation promotes the recovery from impaired motor function after cerebral infarction. However, it still remained largely unknown whether the exercise can induce neurogenesis to reconstruct the neuronal circuit damaged by cerebral infarction. To address this issue, we have examined the role of physical exercise on neurogenesis using photochemically-induced thrombosis (PIT) model in rats. Here, we established motor cortex infarction model and also examined whether the exercise also affects the motor recovery and neurogenesis in this model. One day after operation, exercise group was forced to running exercise using a treadmill for 30 min every day for four weeks. Rota-rod tests revealed that exercise seemed to promote to recovery of motor function during late phase. We performed BrdU labeling experiments which can follow the progeny of newly dividing cells. BrdU immuno-positive cells were detected only around the damaged area in PIT-operated rats. Interestingly, there were more robust BrdU-positive cells in exercise group compared to non-exercise group. These results suggest that physical exercise can promote the motor recovery and the neurogenesis around the damaged areas in rats with unilateral motor cortex infarct.

(COI: No)

P2-058

3D-ultrastructural analysis of the development at the supraspinatus tendon insertion with FIB/SEM tomography

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Introduction: After rotator cuff repair, the repaired tendon-bone insertion is totally different from that of the normal insertion. To regenerate tendon-bone insertion, the morphological development of this region would contribute to the clarification of the pathophysiology in rotator cuff tear. In this study, we analyzed development of the normal tendon-bone insertion in terms of the expression of SOX9/SCX and the 3D ultrastructure.
Materials and methods: 1-, 2-, 3-, 4-week-old SD rats were used. 8 supraspinatus tendon insertion were isolated per each time point. 4 specimens were observed with the fluorescent immunostaining of the SOX9/Scleraxis antibody, and remaining 4 specimens were observed with FIB/SEM tomography.
Results: At 1-week-old insertion, the cells in the insertion region expressed SCX(+)/SOX9(+), the chondroid cells localized between immature collagen bundles. They had many cell processes and connected with each other. As postnatal week passes, the morphology of the cells changed from spherical to the ellipsoidal formation. The number and length of the cell processes were decreased, however, the direction of the cell processes seemed to be extended regularly.
Discussion: The pattern of the expression SOX9/Scrolaxis and the 3D ultrastructural changes in this study would clarify the postnatal development of the normal tendon-bone insertion, may help better understand the pathophysiology of the tendon-bone insertion, especially rotator cuff tears.

(COI: No)

P2-059

Study on structural changes of bone matrix and periostum of femur in growing rats

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Purpose: It is known that structures of bone matrix and periosteum change with growth. Purpose of this study was to investigate relationships between the bone matrix, the periosteum and the bone strength, by observing those structures of the bone matrix and the periosteum, and measuring the bone strength, in growing rats.
Materials and methods: In this study, thirty two male rats (wistar strain, 3, 7 and 13 weeks old) were used as materials, and their femurs were excised after euthanasia. Their right and left femurs were used for measurement of the bone strength and histological analyses, respectively. Both the measurements and the analyses were performed at middle and distal 1/3 portions of diaphysis. Each parameter of the bone strength were measured by bone strength tester, using non-fixation samples, and the structures of bone matrix and periosteum were observed histologically, using decalcified and undecalcified specimens.
Results: Thickness of femoral cortical bone increase wholly, but lamellar structures and thickness of periosteum increased at posterior medial face of femur, with growth. Bone strength indicated higher value at middle portion in the immature stage, but difference of that values between middle and distal portions became lesser with growth.
Conclusion: It was understood that the bone strength increased from middle portion toward distal portion with growth, and the changes were related to thickening of the lamellar bone and the periosteum.

(COI: No)

P2-060

Functional morphology of lumbar spine using X-ray in vivo

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Introduction: Spinal anterior and middle column consists of vertebra and disc. They have a role of weight bearing through vertical direction. Also flexibility and stability are needed especially for bipedal animal. These complex load cause spinal deformity and lifetime morbidity of low back pain is over 80% in humans.
Method: Ninety-five patients with low back pain underwent X-ray photos. Angle of upper and lower edges of vertebra and disc were calculated respectively and analyzed in level, sex and age.
Result: The vertebra-angles at L1, L2, L3, L4 and L5 were 4.2° ± 0.4, 2.1° ± 0.3, 0.8° ± 0.3, -1.3° ± 0.3 and -5.5° ± 0.4, respectively. The disc-angles at L1/2, L2/3, L3/4, L4/5 and L5/S were -2.9° ± 0.3, -4.0° ± 0.3, -6.2° ± 0.3, -7.6° ± 0.4 and -10.9° ± 0.4, respectively. The vertebra-angles were -0.6° ± 0.3 (female) and 0.4° ± 0.3 (male). In terms of disc-angles, there were no statistical differences between female and male. A significant difference in disc and vertebrae was not detected among age groups (20's, 30's, 40's and 50's).
Discussion: Significant level dependence was detected in vivo. The vertebra-angles and disc-angles both showed backward tilting with caudal level and disc showed more retroversion. Female has more lordotic vertebra. It will relate to the difference of pelvic organ. Bone density and muscle volume changes especially in youngers and elderlies. Spinal shape may also be affected with these findings. Although the current study did not show age-dependency, further studies are needed to include larger numbers include other generation.

(COI: No)

P2-061

Age-related changes of elements in the thyroid cartilage of monkey

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The purpose of the present study is to elucidate age-related changes of elements in the thyroid cartilages. The authors determined the elemental contents of the monkey thyroid cartilages by inductively coupled plasma-atomic emission spectrometry (ICP-AES). The monkey subjects consisted of nine rhesus monkeys, one Japanese monkey and three crab eating monkeys, ranging in age from 0.1 to 27 years. It was found that the average content of calcium was 30.9 mg/g in the monkey thyroid cartilages and it is easy to calcification when calcium content of tissue is higher than 10 mg/g. This finding indicates that calcification occurs easily in the monkey thyroid cartilages. Regarding age-related changes of element contents, it was found that the accumulation of calcium increased progressively with aging and showed a sudden rise at 7 years old in the monkey thyroid cartilages. In addition, all the monkeys that calcium content exceeded 20 mg/g were over 7 years old. Likewise, the trend of change of phosphorus was parallel with that of calcium. Therefore, it is likely that the accumulation of calcium and phosphorus may mainly occur after reaching a certain age in the monkey thyroid cartilages. In regard to relationships among the average contents of elements, there were very significant direct correlations among the average contents of calcium, phosphorus, and magnesium in the monkey thyroid cartilages.

(COI: No)

P2-062

Analysis of biological apatite crystal orientation in posterior cortical bone of human maxilla using microbeam X-ray diffractometry

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The purpose of this study was to quantitatively evaluate BMD and BAp crystal orientation using micro-computed tomography(micro-CT) and microbeam X-ray diffractometry in the posterior cortical bone of human maxilla. The intensity and direction of mechanical stresses in both the buccal and lingual area were compared. The maxillary first molar region in Japanese bone samples was designated as the region of interest and BMD and BAp crystal orientation in the buccal and lingual area measured. The results showed no difference in BMD values among regions. BAp crystals were oriented predominantly in the mesiodistal direction in the lingual area and along the direction of masticatory force in the buccal area. These findings suggest that the lingual area exhibits form maintenance such as the dentition maintenance, while in the buccal area alignment takes place in the direction of masticatory force resulting from mechanical stress exerted via the teeth. Qualitative evaluation revealed clear differences between the buccal and lingual area, suggesting that BAp crystal orientation offers a more precise indicator of bone quality than BMD.

(COI: No)

P2-063

Cortical bone water changes in ovariectomized rats during the early postoperative period: objective evaluation using sweep imaging with Fourier transform

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It is important to evaluate bone quality in osteoporosis for the early diagnosis. The purpose of this study was to evaluate the cortical bone signal-to-noise ratio (SNR) in ovariectomized (OVX) rats during the early postoperative period as a method to measure bone quality using the sweep imaging with Fourier transform (SWIFT) technique. Twelve-week-old female Sprague-Dawley rats (n=64) were divided into sham and OVX groups. Preoperative tetracycline was immediately administered subcutaneously to distinguish new cortical bone area, and tibial samples were collected at 2, 4, 8, and 12 weeks postoperatively. Magnetic resonance imaging (MRI) was performed using SWIFT to obtain cross-sectional images of the tibial diaphysis. The cortical bone SNR was calculated. Bone histomorphometry was performed. Histomorphometry findings showed that the new bone area was significantly greater at 8 and 12 weeks postoperatively in the OVX group ($P<0.05$). The SWIFT technique showed that the SNR was significantly higher at 8 and 12 weeks postoperatively in the OVX group ($P<0.05$) and was correlated with the new bone area ($R^2=0.430$). The SWIFT findings suggest that the SWIFT technique may depict early changes in cortical bone quality.

(COI: No)

P2-064

Effects of chewing during prenatal stress on bone microstructure in mice

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Objective: Chronic stress is a risk factor for osteoporosis. Chewing inhibits the stress-induced response. In the present study, we examined the effects of maternal chewing during prenatal stress on bone microstructure of the adult offspring.
Methods: We used three-month-old ddY mice. Animals were divided into control, stress, and stress/chewing groups. Mice in the stress and stress/chewing groups were placed in a restraint tube for 30 minutes, thrice a day from day 12 of pregnancy. Mice in the stress/chewing group allowed to chew on a wooden stick during the same period. The blood corticosterone levels in dams were measured. The male offspring were raised until 5 months old, at which point the trabecular bone in the femur and vertebra was evaluated using micro-CT. The bone formation rate was analyzed and osteoclast number was calculated.
Results: Blood corticosterone levels were significantly higher in the stress group. Chewing under chronic stress prevented the increase of the corticosterone level. Prenatal stress caused a significant reduction of trabecular bone of the offspring. Bone formation rate was decreased and osteoclast number was increased in the stress mice. Chewing under prenatal stress attenuated reduced bone formation and stimulated bone resorption, and improved the trabecular bone loss.
Conclusions: These findings indicate that prenatal stress induced excess secretion of corticosterone, triggered the bone loss. Chewing prevented the increase of corticosterone level, improved the balance of bone formation and bone resorption, ameliorated bone loss induced by prenatal stress.

(COI: No)

P2-065

Histochemical assessment on bone tissue in transgenic mice overexpressing parathyroid hormone-related peptide (PTHrP) driven by type1 collagen promoter

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Purpose: This study aims to elucidate the biological function of parathyroid hormone-related peptide (PTHrP) in bone cells, by examining long bones in PTHrP overexpressing transgenic (Tg) mice.
Materials and Methods: Tg mice overexpressing PTHrP were generated by inserting the PTHrP cDNA downstream type1 collagen promoter specific to osteoblasts. Fetuses at E18 were harvested and immersed in 4% paraformaldehyde solution. The paraffin sections of femora and tibiae were histochemically examined for tissue nonospecific alkaline phosphatase (ALP), tartrate-resistant acid phosphatase (TRAP), and ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1).
Results and discussion: PTHrP Tg mice showed enlarged epiphyseal cartilage with no hypertrophic zone, and therefore, vascular invasion and mixed spicules of cartilage cores and surrounding bone matrix were not observed in the chondro-osseous junction; The resultant bone showed no distinct metaphysis and diaphysis. There were many ALP-positive preosteoblastic cells throughout the Tg bone, but a less number of ENPP1-reactive cells were observed on trabeculae accompanied with a few TRAP-reactive osteoclasts. Taken together, the overexpressed PTHrP appears to affect chondrocyte proliferation and the entry into the hypertrophic phenotype, and also stimulate preosteoblastic proliferation rather than differentiation into osteoblasts.

(COI: No)

P2-066

Effects of mechanical loading on structures of tibial growth plate and primary cancellous bone in rats

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Purpose: Bone trabecula formation is related not only bone addition and resorption at there but also structures of calcified cartilage trabeculas derived from growth plate. We had already recognized that acute bone resorption caused in the early stage of exercise period. Purpose of this study was to investigate structural changes of tibial primary cancellous bone and growth plate accompanied with short-term exercise in growing rats.
Materials and methods: Thirty two male rats (wistar strain, seven weeks old) were used as material, and they were divided into exercise group (EX) and control (CO), randomly. Furthermore, EX was divided into EX2, EX4, EX7 and EX14, according to difference of experimental periods, and CO was also divided into CO2, CO4, CO7 and CO14 similarly. EX2, EX4, EX7 and EX14 performed jumping exercise (45cm height, 100 times per day, every day) for 2, 4, 7 or 14 days. Tibiae were excised from rats after each experimental periods. Bone structures were observed histologically and immunohisotologicaly.
Results: No differences were found in thickness of calcified cartilage trabeculas between every groups. Many osteoclasts were recognized in the early stage of experimental period, but starting portion of bone formation on the bone trabecula got closer to growth plate in EX.
Conclusion: It was suggested that mechanical loading promoted both resorption in the early stage of exercise, but little effects were given to the growth plate.

(COI: No)

P2-067

Comprehensive analysis of osteons in human femoral cortical bones with circularly-polarized microscope

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Background: The increase of the fragile fractures and atypical fractures with osteoporosis in elderly people becomes the problem clinically. The main locus of the bone modification in the cortical bone is osteon. However, there are much unsolved problems on these osteons. We will report a manufactural technique to make polishing specimens from large cortical bones and image analysis measurement.
Method: Subjects were the human femoral bones from the anonymous individuals. We made a 5mm width specimen from the shaft of femur and performed resin embedding. They were polished to make a specimen of 100 μm width manually. Then, the images were taken under the circularly-polarized microscope (CPM). Finally we could analyze the whole section as one large image using a synthetic software.
Results: It was possible to evaluate the distribution patterns of primary and secondary osteons in the whole section. Moreover, all of the osteons could be classified from the aspect of polarization pattern under the CPM.
Discussion: Ascenzi reported that the difference in features of osteon with CPM images depended on the difference in collagen alignment patterns. Qualitative or quantitative analysis of the osteons was possible with CPM in the human femur polishing specimen. All of the osteons in a large axial section could be evaluated with our technique. Hereafter, it will be possible to analyze osteonal structures in cortical bones comprehensively.

(COI: No)

P2-068

Histological examination on bone matrix surrounding osteocytic lacunae after PTH-administration or during lactation of mice fed with low calcium diet

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Introduction: In this study, we have attempted to histologically verify "osteocytic osteolysis" proposed by Bélanger in 1960's., using two mouse models - PTH administer mice and lactating mice with low calcium (Ca) diet.
Materials and Methods: Wild-type (*wt*) ICR and *Rankl*$^{-/-}$ male mice were injected with hPTH (1-34), and then, bone matrix surrounding the osteocytic lacunae was examined under TEM, fluorescence microscopy and confocal laser microscopy. Furthermore, we additionally investigated osteocytic lacunae in lactating mice fed with low Ca diet.
Results and Discussion: Serum Ca concentration was increased at 1 hour after PTH administration in *wt* and *Rankl*$^{-/-}$ mice. At six hours after PTH administration, enlarged osteocytic lacunae were observed mainly in the cortical bone, and von Kossa staining demonstrated broadly demineralized bone matrix surrounding the osteocytes. Under TEM, fragmented collagen fibrils and pieces of mineralized matrices were observed in the enlarged osteocytic lacunae with irregularly-shaped walls. In addition, calcein labeling was seen on the walls of some osteocytic lacunae. In lactating mice with low Ca diet, consistently, the osteocytic lacunae were enlarged, and sometimes labeled with calcein. It seems likely that osteocytes erode the surrounding bone matrix i.e., osteocytic osteolysis, and deposit minerals on their lacunae.

(COI: No)

P2-069

Three-dimensional reconstruction of osteocytic lacunar-canalicular system in murine bone by using FIB-SEM

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Osteocytes extend their thin cytoplasmic processes, by which they communicate with not only neighboring osteocytes but also osteoblasts on bone surface. Thereby, osteocytes establish the cellular network referred to as osteocytic lacunar-canalicular system (OLCS). Focused ion beam-scanning electron microscope (FIB-SEM) is one of the most powerful microscopy for reconstructing the ultrastructural 3D of the objects. In order to clarify the 3D of cellular network of OLCS, we have investigated it using FIB-SEM.
Eight-weeks old ICR mice were fixed with 1/2 Karnovsky solution, and their tibiae were decalcified with 5% EDTA solution. The specimens were post-fixed with OsO4, immersed into an aqueous solution of uranyl acetate and embedded in epoxy resin prior to FIB-SEM observation.
Under FIB-SEM, osteocytes were shown to expand their stout protrusions of the osteocytes' cell bodies, and then, many fine cytoplasmic processes branched off. Some fine processes from the stout cellular protrusion extended horizontally, some turned immediately at a right angle to reach the bone surface, and others arose directly from the osteocytes' bodies and ran perpendicularly to the bone surfaces. Thus, there seemed to be several pathways for cytoplasmic processes of osteocytes to reach the bone surfaces. In summary, FIB-SEM is able to clearly demonstrate the fine ultratrsuctures of 3D reconstruction of osteocytes and their cytoplasmic processes.

(COI: No)

P2-070

PP2A Calpha in osteoblasts controls osteoblast and adipocyte differentiation

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The serine/threonine protein phosphatase 2A (PP2A) participates in regulating many important physiological processes. We examined the role of alpha-isoform of PP2A catalytic subunit (PP2A C α) in osteoblast and adipocyte differentiation. Transgenic mice that specifically express dominant negative PP2A C α in osteoblastic cells showed higher cortical bone mineral density and increase in body weight and adipose tissue of tibia bone marrow. The expression and phosphatase activity of PP2A C α decreased during osteoblast differentiation in osteoblasts. PP2A knockdown cells (shPP2A) were established by infecting lentivirus particles expressing shRNA specific for PP2A C α. shPP2A cells showed accelerated osteoblast differentiation with the upregulation of bone-related genes such as Osterix, Bone sialoprotein, and Osteocalcin. Transcriptional activity of Osterix promoter region was higher in shPP2A cells than that of the control cells, which was controlled by transcription factors Dlx5 and Runx2. To examine the effect of PP2A C α in osteoblasts on adipocyte differentiation, mesenchymal stem C3H10T1/2 cells were co-cultured with shPP2A cells. shPP2A cells showed higher ability to induce adipocyte differentiation and the expression of adipocyte marker genes in C3H10T1/2 cells. Our results indicate that PP2A C α plays an important role in the regulation of bone formation and osteoblast differentiation through the bone-related transcription factors. PP2A C α in osteoblasts is also thought to be involved in controlling adipocyte differentiation.

(COI: No)

P2-071

Histone demethylase Jmjd3 regulates osteoblast differentiation and apoptosis

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Posttranslational histone modifications including methylation are closely linked to regulation of eukaryotic gene expression. Jumonji domain-containing 3 (Jmjd3) is a histone demethylase, which specifically catalyzes the removal of trimethylation of histone H3 at lysine 27 (H3K27me3). In this study, we examined the role of Jmjd3 in osteoblast differentiation and apoptosis. Jmjd3 expression was induced in response to the stimulation of osteoblast differentiation. Silencing of Jmjd3 expression suppressed osteoblast differentiation in vitro and in vivo. Silencing of Jmjd3 decreased the promoter activities of osteoblast-specific transcription factors Runx2 and Osterix and increased the level of H3K27me3 on the promoter regions of these genes. Introduction of the exogenous Runx2 and Osterix partly rescued osteoblast differentiation in the Jmjd3 knockdown cells. On the other hand, knockdown of Jmjd3 in osteoblasts promoted apoptosis in response to serum deprivation. Cleavage of Caspase-3 and PARP induced by serum deprivation, which are mediators of apoptosis, were increased in Jmjd3 knockdown cells. The expression of anti-apoptotic molecule B-cell lymphoma-2, was inhibited in Jmjd3 knockdown cells. The present results indicate that Jmjd3 plays important roles in regulating osteoblast differentiation and apoptosis.

(COI: No)

P2-072

Effect of Nitrogen-containing bisphosphonates on collagen-induced arthritis model mice

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Nitrogen-containing bisphosphonates (NBP) is the strong inhibitor of osteoclastic bone resorption. It has been indicated that one of NBP, alendronate, exacerbated collagen-induced arthritis (CIA) in mice. Minodronate (MIN) is one of another NBP and more strongly inhibit osteoclastic bone resorption than alendronate. However, the effect of MIN on CIA has not been revealed yet. In this study, we examined whether the bone destruction and inflammation induced by CIA was exacerbated by MIN or not. CIA was induced in male DBA/1 mice (8 weeks old) by the sensitization with type II collagen. MIN (4 μmol/kg) was injected once a week from one week before the onset of the first sensitization. At indicated periods, mice were killed and processed for the experiments. MIN-treated group showed a higher clinical arthritic score at every time point than non-treated group. Flow cytometric analysis indicated the enhancement of granulopoiesis in bone marrow in both groups. Granulopoiesis in MIN-treated group was more augmented than non-treated group. Histological analysis indicated the thickening of growth plate and the severe and sustained invasion of granulocytes into the joint cavity in MIN-treated group. Furthermore, Gr-1+ granulocytes directly attached to bone surface. These results indicated that MIN strongly inhibited the physiological bone resorption mediated by osteoclasts and exacerbate inflammation in CIA mice. Further study is necessary to clarify the mechanism of bone destruction in this model.

(COI: No)

P2-073

Critical role of PKR in TNF-α-induced osteoclastogenesis

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Double-stranded RNA-dependent protein kinase (PKR) is also to signal transduction pathways, such as MAPK, NF-κB and Smad. TNF-α, one of the inflammatory cytokines, induces osteoclast differentiation and plays a role in progression of inflammatory bone destruction. However, it is unknown about the roles of PKR in TNF-α-induced osteoclast differentiation. Therefore, the present study was undertaken to clarify the role of PKR in TNF-α-induced osteoclastogenesis. The expression of PKR in RAW264.7 cells increased by TNF-α. The TNF-α-induced osteoclast differentiation was markedly suppressed by the pre-treatment of 2-aminopurine (2AP) and PKR inhibitor, a specific inhibitor of PKR as well as PKR siRNA. PKR inhibition also suppressed bone resorption activity. PKR siRNA or 2AP suppressed the TNF-α-induced activation of NF-κB and MAPK in osteoclast precursor. Translocation of NF-κB to nucleus was also suppressed by 2AP. 2AP inhibited the TNF-α-induced expression of NFATc1 and c-fos, master transcription factors in osteoclastogenesis. TNF-α-induced nuclear translocation of NFATc1 in mature osteoclasts was clearly inhibited by the 2AP treatment. The PKR inhibition decreased the TNF-α-induced osteoclast formation and bone resorption in mouse calvaria. Collectively, these results demonstrated that PKR regulates TNF-α-induced osteoclast differentiation and suggested to be a pivotal therapeutic target for TNF-α-induced bone destruction.

(COI: No)

P2-074

Chondroitin sulfate inhibits osteoclast differentiation and bone resorption activity, and improve bone metabolism

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Chondroitin sulfate (CS) is a kind of glycosaminoglycan, which existed in the bone extracellular matrix. In present study, we investigated the osteoclast inhibition ability compared to with or without sulfation groups in CS and the mechanism how CS inhibit osteoclast differentiation in vitro. Both sulfated-CS (s-CS) and non-sulfated-CS (ns-CS) were significantly inhibited the increase of tartrate-resistance acid phosphatase (TRAP)-positive multinucleated cells and had negative effect on increasing osteoclast size. Pit formation assay revealed that CSs also suppressed bone resorption activity. Whereas chondroitinase ABC-digested CS did not show the inhibition activity of osteoclast differentiation and function. Quartz-crystal microbalance analysis clarified that CS possessed the binding ability to receptor activator of NF-κB ligand (RANKL) and interrupted binding of RANKL to RANK. Furthermore, CS reduced the phosphorylation of extracellular signal-regulated kinase in pre-osteoclast cells shown by flow cytometrical analysis. These in vitro results indicate that CS suppresses both osteoclastogenesis by binging to RANKL and osteoclast size increase. Moreover, CS inhibits RANKL-induced signal pathway, which results in decrease of the osteoclast bone resorption area. Moreover, we treated CS to osteoporosis model mouse for 8 week by intraperitoneal administration. The osteoclast activity of the animal was downregulated and osteogenesis in bone was promoted. These results suggest that CS have a potential to improve bone metabolism in vivo.

(COI: No)

P2-075

Three-dimensional morphology of Golgi apparatus of osteoclasts by scanning electron microscopy using OsO_4 maceration method

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Introduction: Osteoclasts have highly-developed Golgi apparatus around their nuclei. Although two-dimensional structure of Golgi apparatus in osteoclasts have been reported, its three-dimensional structure is still veiled. This study was designed to elucidate the three-dimensional structure of the Golgi apparatus in osteoclasts by scanning electron microscopy using OsO_4maceration method.

Materials and methods: Eight-week-old Wistar rats were perfused with a mixture of 0.5% glutaraldehyde and 0.5% paraformaldehyde. The femora were dissected out, sagittally freeze-cracked, and post-fixed with 1% OsO_4. The specimens were then immersed in 0.1% OsO_4 for 10-12 days at 20℃ according to Tanaka et al (1984). They were dehydrated, critical point-dried, and coated with OsO_4 or platinum-palladium.

Results and discussion: Actively bone-resorbing osteoclasts on femoral trabeculae were clearly identified by multi-nuclei, a lot of vacuoles and mitochondria, and well-developed ruffled border. The Golgi apparatus in the vicinity of the nuclei consisted of 4-5 layers of cisterns and small vesicles. The cis-most cistern facing the nucleus revealed the meshwork with regularly-arranged small pores. In contrast, the trans-most cistern demonstrated a plate-like structure with a few pores. The network of such Golgi apparatus widely covered the nuclear surface, with leaving focal fenestrations. These findings suggest that the Golgi apparatus in osteoclasts almost encompasses the entire surfaces of nuclei like a basket.

(COI: No)

P2-076

Expression of MicroRNAs in the Extracellular Vesicles during Osteoclastogenesis

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MicroRNAs (miRNAs) are small, non-coding RNAs that are involved in various biological processes, including cellular differentiation, proliferation, apoptosis, and organ development. We previously profiled miRNA expression during osteoclastogenesis using microarrays. Recently, the presence of miRNAs in extracellular vesicles was reported. It is not known whether osteoclasts secrete extracellular vesicles containing miRNAs. We investigated miRNA expression in the extracellular vesicles in conditioned medium of cultured osteoclasts using RT-PCR. Specifically, we investigated eight miRNAs deemed important for osteoclastogenesis in our previous study: let-7e, miR-21, miR-33, miR-155, miR-210, miR-223, miR-378, and miR-1224. Of these, the expression levels of miR-378, miR-210, and miR-21 were very high, while no significant miR-33 or miR-1224 expression was detected. These results suggest that osteoclasts secrete extracellular vesicles containing specific miRNAs, but that they do not contain the entire set of intracellular miRNAs.

(COI: No)

P2-077

Lidocaine induces ROCK-dependent membrane blebbing associated with subsequent cell death in rabbit articular chondrocytes

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Local anesthetics are administered intraarticularly for pain control in orthopedic clinics and surgeries. Previous studies have shown that local anesthetics can be toxic to chondrocytes, although the underlying mechanism remains unclear. The present study was undertaken to investigate the cellular mechanisms associated with lidocaine-induced toxicity to articular chondrocytes. Isolated rabbit articular chondrocytes were exposed to lidocaine and monitored under a light microscope. Clinical concentrations of lidocaine caused membrane blebbing. ROCK inhibitors Y-27632 and fasudil completely prevented the lidocaine-induced blebbing, suggesting that ROCK activation is required for the bleb formation. The GTP-bound RhoA level was significantly increased (3.01 ± 0.76 folds, $P < 0.0001$) by 20-min treatment with 10 mM lidocaine, suggesting that RhoA activation is involved in ROCK activation. Chondrocyte viability significantly decreased to $17.6 \pm 5.7\%$ after 1-hour exposure to 30 mM lidocaine, compared with the control viability of $94.8 \pm 2.4\%$ ($P < 0.0001$). Pretreatment with 10 μM Y-27632 or 100 μM fasudil attenuated the lidocaine induced-cytotoxicity ($49.4 \pm 12.5\%$ and $47.2 \pm 9.1\%$ viability respectively, $P < 0.0001$). These findings show that lidocaine induces a cytotoxic effect on chondrocytes through a mechanism involving membrane bleb formation and ROCK activation and that caution should be taken when administering lidocaine intraarticularly.

(COI: No)

P2-078

Effect of glutathione on TNFα-induced osteoclast differentiation in murine bone marrow-derived macrophages

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Osteoclast differentiation is regulated by TNF α and RANKL in inflammatory bone destruction in rheumatoid arthritis; and reactive oxygen species (ROS) have been shown to act as a signaling molecule in TNF α signaling suggesting that ROS play a role in TNF α-mediated osteoclast differentiation. Glutathione is an intra- and extra-cellular antioxidant against oxidative stress in inflammation. Therefore, we have investigated the effect of glutathione on TNF α-induced osteoclast formation using murine bone marrow-derived macrophages. Glutathione significantly stimulated the TNF α-induced osteoclast formation and buthionine sulfoximine, an inhibitor of glutathione synthesis, suppressed the TNF α-induced osteoclast formation. Glutathione facilitated the protein expression and nuclear translocation of NFATc1, a master regulator of osteoclastogenesis as well. In time-lapse analysis, glutathione increased the incidence of TNF α-induced cell fusion of osteoclasts. Furthermore, N-acetylcysteine, a substrate of glutathione synthesis, also stimulated osteoclast formation and NFATc1 nuclear translocation. Thus, these results suggest that glutathione is positive regulator of TNF α-stimulated osteoclast differentiation.

(COI: No)

P2-079

Ultrastructural assessment for biological function of vascular endothelial cells at chondro-osseous junction

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Purpose: Chondro-osseous junction is the site of vascular invasion in the process of endochondral ossification, which replaces epiphyseal cartilage with bone. Osteoclasts, bone-resorbing cells, are not intrinsic for endochondral ossification, because the long bones of osteoclast-less mice can grow longitudinally. In this study, we have histologically examined vascular invasion of endothelial cells at the chondro-osseous junction.
Materials and Methods: ICR mice at eight weeks of age were perfused with 1/2 Karnovsky solution from the left ventricle, and then, tibiae were extracted and immersed in the same fixatives. The specimens were decalcified with 5% EDTA and embedded into epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate prior to TEM observation.
Results and discussion: At the tibial chondro-osseous junction, the cytoplasmic processes of vascular endothelial cells were shown to extend into the transverse partitions of columns of hypertrophic chondrocytes. Hypertrophic chondrocytes adjacent to such vascular invasion seemed to be intact featuring normal cell organelles and enlarged cell bodies. But, some cell debris was observed in blood vessels close to the junction, and cells neighboring the endothelial cells possessed large secondary lysosomes. Thus, the cellular interplay between the endothelial cells and the surrounding cells seems to be essential for vascular invasion during endochondral ossification.
(COI: No)

P2-080

Detection of early changes after growth plate injury

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Growth plate injuries can cause a premature closure of the growth plate, and may lead to limb shortening or deformity. The purpose of this study was to elucidate the relationship between the size of the growth plate injury and the timing of the beginning of physeal growth disturbance in non-injured regions. Thirty-two 5-week-old male Japanese white rabbits were used. Injuries were made to the central region of the proximal growth plate of the right tibia, by using a 3.0-mm drill (3.0 mm group) and a 1.2-mm drill (1.2 mm group). Left tibia was used as the control. Imaging of the growth plates was performed at 1, 4, 8, 10 and 12 weeks after the injury, by using MRI. Findings at 10 and 12 weeks after injury in the 3.0mm group showed that the growth plates in non-injured regions were significantly reduced on both the medial and lateral sides, compared to those found in the control. Findings in the 1.2mm group showed that at 12 weeks after injury, growth plates in non-injured regions were significantly reduced on both the medial and lateral sides. Tibia length in both groups was significantly shorter than that found in controls at 12 weeks after injury. These findings showed that the size of the growth plate injury was associated with the timing of the beginning of physeal growth disturbance in non-injured regions.
(COI: No)

P2-081

The roles of promyelocytic leukaemia zinc finger (PLZF) in Glucocorticoid-induced cell cycle arrest in a chondrogenic progenitor cells, ATDC5

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Glucocorticoids inhibit long-bone growth by suppressing the proliferation of chondrocytes in growth-plate. However, the mechanisms by which glucocorticoids induce cell cycle arrest or apoptosis in chondrocytes are not well understood. In this study, we investigated the expression of the glucocorticoid-induced transcription factor promyelocytic leukemia zinc-finger (PLZF) during chondrocyte differentiation using a chondrogenic progenitor cell line, ATDC5. PLZF expression was up-regulated during chondrocyte differentiation. Furthermore, treatment with a GR antagonist showed that glucocorticoid-induced up-regulation of PLZF is mediated by the GR. To elucidate the roles of PLZF in chondrocyte proliferation, we transfected ATDC5 cells with the PLZF gene and found that PLZF overexpression suppressed proliferation by up-regulation of a cyclin-dependent kinase inhibitor, p21WAF1/CIP1 (p21) expression. In contrast, PLZF short hairpin RNA (shRNA) suppressed differentiation into hypertrophic chondrocyte, and promoted cell cycle progression by down-regulation of type X collagen and p21 mRNA expression. Furthermore, PLZF shRNA attenuated glucocorticoid-induced cell cycle arrest by down-regulation of p21 mRNA expression. These results clearly indicate that physiological levels of PLZF promote hypertrophic phenotypes, whereas excess levels of PLZF are involved in glucocorticoid-induced cell cycle arrest by regulation of CDK inhibitor in chondrocytes.
(COI: No)

P2-082

Infruence by excess and deficiency of retinoic acids on septoclasts in the epiphyseal plate of mice

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Objective: We found that the septoclast, a mononuclear cartilage-resobing cell, express exclusively epidermal type-fatty acid-binding protein (E-FABP) in the epiphyseal plate of ddY mice. E-FABP has high affinity for retinoic acids (RAs) as well as fatty acids. We investigated the effects of both excess and deficiency of RAs on septoclasts.
Methods: RA-excess ddY mice were given a single oral dose of 300 mg/kg RAs in soybean oil at 4-weeks old, and RA-deficient mice received the vitamin A-free diet from weaning to 9-weeks old. Frozen sections of the epiphyseal plate were obtained from the proximal tibia of these mice. Immunohistochemistry, immunoelectron microscopicopy and 3-D analysis were performed to detect E-FABP-positive septoclasts. Double immunohistochemistry of E-FABP and peroxisome proliferator-activated receptors (PPARs) were performed.
Results: In both RA-excess and -deficient mice, number of septoclasts decreased compared with control ones. E-FABP-positive cells posessed reduced number and shortened cell processes projecting to the transverse septa lining the chondro-osseous junction. E-FABP-positive septoclasts were simultaneously immunostained with PPARβ/δ.
Discussion: These results suggest that RAs are incorporated by septoclasts and used with PPARβ/δ to regulate cartilage-resorption activity of septoclasts. RAs are important for morphological maintenance of septoclasts.
(COI: No)

P2-083

Suppressive activity of glucosamine on osteopontin-induced nitric oxide (NO) production from human synoviocytes in vitro

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Objective: The present study was designed to examine the influence of glucosamine hydrochloride (GH) on the ability of synoviocytes to produce nitric oxide (NO), which is the important effector molecule in the development of osteoarthritis, in response to osteopontin (OPN) stimulation in vitro.
Methods: Synoviocytes (5 x 10^5 cells/ml) derived from osteoarthritis patients were stimulated with 330 ng/ml OPN in the presence of various concentrations of GH for 24 hours. The levels of NO in culture supernatants was examined by NO_2/NO_3 assay kits. To examine the influence of GH on transcription factor, NF-κB, activation and iNOS mRNA expression, synoviocytes (5 x 10^5 cells/ml) were also cultured in a similar manner for 4 and 12 hours, respectively. The levels of both mRNA expression and transcription factor activation were measured by ELISA.
Results: Addition of GH into cell cultures caused the suppression of OPN-induced NO production from synoviocytes. The minimum concentration that caused significant suppression of NO production was 1.0 mg/ml. GH at more than 1.0 mg/ml also inhibited iNOS mRNA expression and NF-κB activation, which were increased by OPN stimulation in synoviocytes.
Conclusion: These results strongly suggest that GH favorably modify the clinical condition of osteoarthritis patients through the suppression of NO production from synoviocytes.
(COI: No)

P2-084

Fourier transform infrared spectroscopy and atomic force microscope observations of regenerative tendon biomaterial

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In this study, we used Fourier transform infrared (FT-IR) spectroscopy and atomic force microscopy (AFM) to analyze tendon gel, a biomaterial produced from injured tendons in mice, before and after applying tensile stress to obtain quantitative information regarding the mechanism of regeneration in injured Achilles' tendons. AFM revealed periodic striations of collagen fibers aligned along the tensile direction in the tendon gel. The FT-IR spectrum showed that cross-linking of collagen molecules began in the tendon gel after applying tensile stress. Thus, FT-IR and AFM are effective techniques for quantitative evaluation of the regenerative process in tendon biomaterials.
(COI: No)

P2-085

Three-dimensional construction of the twisted structure of the Achilles tendon: focusing on twisted angle

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Many studies have investigated the twisted structure of the Achilles tendon (AT) for many years. However, no consensus for twisted angle has been reached by the differences in measurement methods or reference axes. The purpose of this study was to three-dimensionally construct the structure of each tendon fiber bundle constituting the AT and to clarify twisted angles in three dimensions (3D). Three types of AT twists categorized by our previous study were used; one each of Type I (least), Type II (moderate) and Type III (extreme) (3 legs). Using a Microscribe device, fascicles that originate from the medial head of the gastrocnemius (MG), lateral head of the gastrocnemius (LG), soleus muscle (Sol), and the calcaneal tuberosity (4 points) were digitized to construct 3-dimensional models. An absolute coordinate system was created on the basis of an arbitrarily determined rotation center of the calcaneal tuber, and the angles of each fiber bundle to the three axes of the absolute coordinate system were calculated. SCILAB-5.5.0 was used for the analysis. Each type of AT twists was as follows; for Type I, lateral axis: 86.1 ± 3.3°, vertical axis: 173 ± 2.4° and longitudinal axis: 92.6 ± 1.7°; for Type II, 93 ± 3°, 175 ± 2° and 91 ± 1°; for Type III, 70 ± 4°, 60 ± 4° and 90 ± 1°. This study demonstrated that the AT has a three-dimensional twisted structure. Further studies should be needed by calculating twisted angles to the joint axes such as the talocrural or the talocalcaneal joints.

(COI: No)

P2-086

Immunolocalization of E-FABP in Meckel's Cartilage of Mice

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Objective: Septoclasts, mononuclear cartilage-resorbing cells in the epiphyseal growth plate, express epidermal-type fatty acid binding protein (E-FABP or FABP5). They locate at the chondro-osseous junction and project several long processes onto the uncalcified cartilage matrix. Meckel's cartilage (MC) develops earlier than mandibular bone and degenerates before birth except for the portion forming the ear ossicles. The present study aims to clarify the localization of E-FABP-positive septoclasts during the degeneration of MC.
Methods: Embryos at 15th day (E15), E16, E17 and E18 of ddY mice were fixed by 4% paraformaldehyde and prepared for frozen serial sections. Immunohistochemical procedures were performed using a specific polyclonal antibody against mouse E-FABP.
Results: E-FABP-positive small, spindle-shaped cells were initially detected at the junction of hypertrophied MC and fibrous connective tissues in E16, and increased in number to E17 and E18. E-FABP-positive cells with or without cell processes were located interior of opened hypertrophic cartilage lacunae. No cells expressing E-FABP were found at any healthy portions of MC comprising resting or proliferating chondorocytes in mice of E15 to18.
Discussion: These results suggest that septoclasts participate in the resorption of MC during its degeneration. Fatty acids and relative lipids affinity to E-FABP may regulate MC degeneration.

(COI: No)

P2-087

Cocktails of certain growth factors that induce differentiation of periodontal ligament cells

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Object: To establish a model *in vitro* that demonstrates the differentiation of periodontal ligament (PDL) mesenchymal cells into osteoblasts(Ob)-like cells to regenerate alveolar bone for the implant dentistry.
Materials and Methods: We isolated mesenchymal cells from the rat molar PDL (Wistar rats; male, 120 g; n=40). The cells were cultured until confluence (37 ℃, humid air with 5% CO2), and then maintained in MINIMUM ESSENTIAL MEDIUM EAGLE (SIGMA; added with AA + 2mM L-glutamine) by adding different combinations of growth factors for 7, 14, 17 or 21 days. Growth factors of 100/200 ng/ml BMP-2, 100/200 nm dexamethasone (DEX), 100 ng/ml IGF-1 and 50 ng/ml bFGF were used. Cell proliferation and differentiation were evaluated by cell counting and alizarin red S staining for the osteogenic cultures. Moreover, we examined the existence of anti-Runx2 and anti-osterix immunoreactive (IR) cells in the cultures by immunohistochemistry.
Results: 1) The 100 ng/ml BMP-2 + 100 nM DEX group: the alizarin red S-stained cells significantly increased in number at the day 14 of culture. 2) The 50 ng/ml bFGF group: the cells were significantly proliferated, but were not induced to differentiate into Ob-like cells until the day 21. 3) The 100 ng/ml IGF-1 group: the cells were induced to differentiate into Ob-like cells after 17 days of culture.
Conclusion: A cocktail of BMP-2 and IGF-1 significantly induced the PDL mesenchymal cells to differentiate into osteogenic Ob-like cells.

(COI: No)

P2-088

Micro-CT-based volume rendering of the lingual muscle in developmental- and postnatal-stage mice and in other animals

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Purpose: The writers already announced that a method to observe a soft tissue in three-dimension (3D) using MicroCT was effective. However, in the case of tongue muscles, the conventional image processing of the 3D information stops in the display example of a tomogram, and a simple 3D image. Only an organ example in the developmental stage was reported in the data. We prepared a mouse for the developmental stage. A postnatal mouse, a cattle tongue, a toad tongue, a tortoise, and a bird in normal laboratory levels were used as the examples this time. The fructification that tried the volume rendering of the muscle unit is reported.
Method: Each tissue was fixed with a 4% neutrality Formalin for one week. The tissue was then freeze-dried according to reduction to a single unit after the decalcification with a Plank-Rychlo liquid for 12 hours. The other part of the tissue was fixed with a 1% osmic acid for 12 hours, and a paraffin preparation was made according to reduction to a single unit. These preparations were photographed in MicroCT. The image processing software performed the image processing.
Fructification: 1. The lingual muscles were showed by the volume rendering images. 2. A lingual muscle in a phylogenetic viewpoint enabled the observation. 3. Beneficial information to study the murine lingual muscle morphosis was obtained.

(COI: No)

P2-089

Effects of mild hyperbaric oxygen on macrophage infiltration during rat skeletal muscle regeneration

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Although hyperbaric oxygen at over 2 atmospheres absolute with 100% O_2 promotes healing of skeletal muscle injury, it is not clear whether mild hyperbaric oxygen less than 2 atmospheres absolute with normal air is equally effective. The purpose of the present study was to investigate the impact of hyperbaric oxygen at 1.25 atmospheres absolute with normal air on muscle regeneration. The tibialis anterior muscle of male Wistar rats was injured by injection of bupivacaine hydrochloride, and rats were randomly assigned to a hyperbaric oxygen experimental group or to a non-hyperbaric oxygen control group. Immediately after the injection, rats were exposed to hyperbaric oxygen. The cross-sectional area of centrally-nucleated muscle fibers was significantly larger in hyperbaric oxygen group than in control group at the early phase after injury. The number of CD68 or CD206 positive cells and the expression levels of TNA-α and IL-10 mRNA were significantly higher in hyperbaric oxygen group than in control group at the early phase after injury. The number of Pax7 and MyoD, or MyoD and myogenin positive nuclei and the expression level of these proteins were significantly higher in hyperbaric oxygen group than in control group 5 days after injury. These results suggest that mild hyperbaric oxygen promotes skeletal muscle regeneration after injury, possibly due to reduced hypoxic conditions leading to accelerated macrophage infiltration and phenotype transition.

(COI: No)

P2-090

Quantitative observation of flexor hallucis longus muscle by using ultrasonograph

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Using ultrasonograph, we have observed cross-sectional image of the flexor hallucis longus muscle at rest, and have investigated sex difference in six healthy male and six healthy female subjects. We confirmed the presence and location of this muscle in vertical direction, and showed that this muscle existed almost middle third of the lower leg. Besides, this muscle of male was longer than that of female in vertical direction. Cross-sectional image of flexor hallucis longus muscle was the most clearly viewed at the central part of the lower leg, but the border of this muscle and surrounding tissues was not constantly apparent. We therefore attempted to quantify the muscle not by area, but by length. We determined three distinguishable points on cross-sectional image of this muscle as landmarks. Then, we measured medio-lateral length and antero-posterior length in cross-sectional image based on these three landmarks. In all subjects, the flexor hallucis longus muscle was longer in medio-lateral direction than in antero-posterior direction. This muscle of male showed a tendency to be longer than that of female in vertical length and horizontal direction. No sex difference was showed in antero-posterior length.

(COI: No)

P2-091

Interaction between supramolecular organization of sarcomeric proteins and myowater revealed with heat denaturation

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Magnetic resonance (MR) images reflect not only water content, but also water states in the tissue. Details of each water state are, however, not clarified yet. In skeletal muscle, MR distinguishes five water states whose localization has been clarified taking advantage of well-organized crystalline sarcomere structure. As has been reported for monomeric (G-) and polymerized filamentous (F-) actin (Wazawa, 2011), proteins develop additional mode of interaction with surrounding water molecules with the order of supramolecular organization. With this view in mind, we observed heat capacity of skeletal muscle (prepared from sartorius muscle of *Rana Catesbeiana*) using differential scanning calorimetry (DSC). With increase in temperature, water molecules released from any intermolecular interaction absorbed additional heat to form an endothermic peak as in the case of melting of ice. Muscle preparation showed endothermic peaks at -25, -22, 0, 45 and 63℃. Each of the peaks at 45 and 63℃ would reflect irreversible denaturation at specific higher-order structure as general heat denaturation of proteins does. Correspondingly, a 45℃ peak irreversibly diminished later -25℃ peaks, and a 63℃ peak diminished later -22℃ peaks with an increase in the integrated heat capacity from -80℃ to 20℃. These results suggest that molecular or supramolecular organization of muscle sarcomere that is subject to heat denaturation significantly affects their interaction with myowater causing substantial endothermic peaks with temperature.

(COI: No)

P2-092

Non-invasive evaluation of skeletal muscle using the wavelet analysis

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The mechanomyogram (MMG) reflect the "mechanical activity of muscle" is a slight vibration associated with muscle contraction, unlike the EMG to record the electrical activity of muscle contraction, muscle sound is, to exercise it is obtained by recording a fine vibration due to skeletal muscle contraction by measuring by placing a vibration sensor at a site file. However, the physiological phenomena of muscle properties reflected by the features of the MMG and electromyography(EMG) signals, and mechanisms of contraction are still not fully clear during movements. The non-invasive method using the wavelet transform will gave a useful analysis for low sound of masticatory muscle with complex noisy background during movements. Therefore we try to accomplish analysis the MMG in masticatory function elucidation by performing the position change of the masticatory muscles of voluntary movement. The distance measurement by laser, MMG and EMG were measured to compare skeletal muscle (biceps brachii) and masticatory muscle in this study. As a result, the sound is found in two areas lower the difference in frequency, moreover, depends on the type of muscle, the present analysis methods muscle contraction, the movement of the muscle fibers and fascia seen when tension reflects the function of the muscle possibility has been suggested.

(COI: No)

P2-093

Characteristics of time course of gene expression in muscle atrophy in cast- immobilized rat model

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We analyzed relevant gene expression while atrophic change in model by cast immobilization on rats. According to our previous study we revealed that the blood flow rapidly decreased in the immobilized leg, and then gradually decrease in muscle weight ant muscle fiber cross sectional area occurred observed after the immobilization applied. This trend was found especially in SO-Fiber (Type-I) rich muscle such as soleus muscle. In this study, we experimented on rat model to simulate muscle atrophy, and analyzed the gene expression pattern in the model utilizing immobilization. Male Fisher rats were used in this study. The right hind-limbs of Experiment group rats were immobilized in a plaster cast (left contra-lateral hind-limbs were analyzed as experimental controls). Gene expression was analyzed encyclopedically in DNA micro array at the following time points; 6 hours, 1 day, 4 days, 10 days after the procedure. We focused on 86 probes which showed significant difference between control and experimented side at each sampling point. Then we searched the relationship of the found probes to their roles either of blood flow or muscle volume. It was revealed that blood flow probes had metabolism regulation feature and muscle volume, structure building feature.

From the analysis it seemed that decrease in blood flow induce suppression of metabolism regulation genes, and then gradually suppress structure related genes to lead morphological change.

(COI: No)

P2-094

Shortening velocity of knee extensor in frog; the effects of the contraction of other lower limb muscles on it

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The present study was investigated to know how the shortening velocity of knee extensor was influenced by the contraction of other lower-limb muscles. We measured the force - velocity relationship, *in vivo*, in whole muscle preparations in knee extensor, triceps femoris muscle (TFM), of the frog, *Rana catesbeiana*. TFM consists of three muscles, rectus femoris (RFM), vastus medialis (VMM) and vastus lateralis (VLM). Frogs were anesthetized and the four kinds of preparations mentioned below were made. Their isotonic shortening velocities were measured at various steps of load at 20 ± 0.5 °C. 1) In the first preparations, all the muscles of thigh were contracted by stimulating sciatic nerve. 2) In the second ones, the sciatic nerve to VMM and VLM was exclusively stimulated by cutting all other branches. 3) In the third ones, the sciatic nerve to RFM, VMM and VLM was exclusively stimulated as in 2). 4) In the fourth ones, the sciatic nerve to hamstrings, VMM and VLM were exclusively stimulated as well. The maximum shortening velocity in 1) was the fastest among four preparations, and the shortening velocity at heavier load (0.5-0.9) was about twice as fast as the others. And the output of power in 1) was also the largest. These results indicate that the interaction between muscle contractions have remarkable influence on shortening velocity and power output, suggesting that interaction between muscle would be able to produce higher power.

(COI: No)

P2-095

Medial Pterygoid initiated the Growth of the Mandible through Premature Muscle Contraction

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Craniofacial growth is influenced by the interaction of muscle and bone tissues. The medial pterygoid is one of the muscles of mastication attached to the mandible. The purpose of the study was to investigate the relation between the medial pterygoid and mandible during embryogenesis. Specimens were prepared from thirty fetal mice at embryonic day (ED) 12, 13 and 14. Slides were stained with hematoxylin and eosin and observed under the light microscope. Immunohistochemistry using desmin, a muscle specific marker, as well as tenomodulin, a tendon specific marker, were also carried out. Results showed that at ED 12, the medial and lateral pterygoid and tensor veli palatini were adjacent to one another. At ED 13, the mandible started to form while the medial pterygoid moved towards the developing mandible. At ED 14, the palatine shelves were also seen in a horizontal position. Over time, desmin localization was observed at myotendinous junctions in between the medial pterygoid and Meckel's cartilage as well as in between the medial pterygoid and mandible and finally in the center of the muscle. Tenomodulin first appeared at ED 13 and had formed spaced linear arrays at either end of the muscle fiber by ED 14. The results suggest that although the muscles of mastication were still immature, the premature contraction of medial pterygoid and the positional relationship provide a dynamic change between the development growth of the mandible and the start of the fusion of the secondary palate.

(COI: No)

P2-096

Epac1 mediates masseter muscle hypertrophy induced by chronic stimulation of β_2-adrenoceptor

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To further elucidate the role of Epac (exchange protein directory activated by cAMP) in the signaling mechanisms responsible for the β_2-adrenoceptor (β_2-AR)-mediated phenotypic changes of skeletal muscle, we examined the effects of chronic stimulation of β_2-AR with clenbuterol (CB) (i.p., 2mg/kg/day for 3 weeks), a β_2-AR agonist, on myofiber cross-sectional area (CSA) and fiber-type composition in masseter muscle (the principal jaw closer in rodents) of wild-type (WT) and Epac1-null (Epac1KO) mice. Masseter muscle mass and myofiber CSA were significantly increased by the CB treatment in WT while not in Epac1KO, demonstrating that Epac1 is involved in β_2-AR signaling promoting muscle hypertrophy. In contrast, the CB treatment significantly increased the proportion of type-IIB fiber at the expense of that of type-IID/X in both WT and Epac1KO, indicating that Epac1 did not mediate the CB-induced slow-to-fast fiber-type transition. The inhibition of the CB-induced masseter hypertrophy by Epac1 disruption was associated with the suppression of CB-induced phosphorylation of Akt and its downstream molecules, S6K1, 4E-BP1 and GSK-3β, as well as CaMKII and its target, HDAC4, a negative regulator of MEF2. These results suggest that Epac1 plays important roles in the β_2-AR-mediated masseter muscle hypertrophy without affecting the slow-to-fast fiber-type transition, potentially through subsequent activation of both Akt and CaMKII/HDAC4 signaling pathways.

(COI: No)

P2-097

TRPV1 channel regulates skeletal muscle regeneration and satellite cell differentiation

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PURPOSE: The transient receptor vanilloid type 1 channel (TRPV1) is a member of non-selective cationic channel family. The activation of TRPV1 induces the influx of Ca^{2+}. Previous studies have demonstrated that Ca^{2+} entry is required for myogenic differentiation and satellite cell (SC) fusion. However, the role of TRPV1 in muscle cell and SC is unknown. The purpose of this study was to determine the role of TRPV1 on muscle regeneration and SC functions.
METHODS: SCs were isolated from male C57BL/6J mice (10 weeks). Capsaicin (cap), TRPV1 agonist, (1 μM) was added to culture media. We confirmed that siRNA transfection silenced TRPV1 gene expression. All animals were injured by the injection of cardiotoxin (CTX) into tibialis anterior (TA) muscles to induce muscle damage in vivo study. Daily injection of cap (1 μM) or physiological saline (con) was performed 8 consecutive days from 3 days before and 5 days after the CTX injection. TA muscles were sampled to analyze regeneration by H&E staining at 5 days after the injection.
RESULTS: We observed that cap induced a significant increase in expression of two makers of SC differentiation. TRPV1 silencing reduced the fusion index. In addition, we observed that exogenous addition of IL-4 was able to restore normal fusion in TRPV1 silenced SCs. The fiber area of the muscles with central nuclei isolated from the cap group was significantly larger than that of muscles from the con group.
CONCLUSIONS: TRPV1 may play critical roles in SC differentiation and skeletal muscle regeneration.
(COI: No)

P2-098

Inhibition of junctional membrane-targeting of skeletal muscle L-type calcium channel by point mutation in the junctophilin-binding domain of $Ca_V1.1$ subunit

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Skeletal muscle L-type calcium channels (LTCC) are specifically localized to the junctional membrane (JM) where the sarcolemma are closely apposed to the sarcoplasmic reticulum, and form a functional complex with ryanodine receptors. Although the targeting of LTCC is critical for efficient excitation-contraction coupling, its molecular mechanism has not been clarified. We previously showed that junctophilins (JP) regulate the proper JM-targeting and function of LTCC through binding to $Ca_V1.1$ subunits. Moreover, a GST-pull down study showed that the JP-binding domain (JBD) is located in 1595-1606 amino acid residues in the C-terminus of $Ca_V1.1$. In this study, we first conducted alanine scanning to the recombinant GST-JBD fusion protein, and examined changes in the binding property of the protein to JPs by the pull down assay. Whereas the binding to JPs is preserved by the alanine substitution of E1595, R1596, and G1606, mutations of other residues in JBD attenuated the binding. We next prepared $Ca_V1.1$_R1596A and $Ca_V1.1$_R1600A and transiently expressed them in GLT myotubes. Immunocytochemical analysis revealed that the JM-targeting rate of $Ca_V1.1$_R1600A but not $Ca_V1.1$_R1596A was significantly reduced compared to the wild type. These results suggested that JBD in C-terminus of $Ca_V1.1$ contributes to the proper JM-targeting of skeletal muscle LTCC.
(COI: No)

P2-099

In vitro effects of K^+ ATP channel agonist on LES tone in rats

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The lower esophageal sphincter (LES) is a specialized region of the esophageal circular smooth muscle that allows passages of a swallowed bolus to the stomach. Functional disorder of an esophagus such as achalasia displays a diminished peristalsis in lower esophagus. It has been reported that nitric oxide plays a major role in LES relaxation. However, the detailed mechanism involved in the regulation of LES activity is still elusive. Nicorandil possesses dual properties of a nitrate and K^+ ATP channel agonist, and is known to reduce LES tone. The present study was carried out to clarify the mechanisms underlying the effects of nicorandil on LES. In particular, possible involvement of K^+ ATP channel was investigated. LES tissues of rats were placed in a standard organ bath and activities were recorded using the software Chart Pro v 4.0. After contraction with carbachol, K^+ ATP channel agonists (nicrandil, pinacidil, diazoxide) were added directly to the tissue bath. Ant they caused a significant relaxation of the LES. Further, the K^+ APT ATP channel blocker glibenclamide prevented the LES relaxation caused nicorandil. On the other hand, the nitoric oxide synthase inhibitor L-NAME, the guanylate cyclase inhibitor ODQ and BKCa channel blocker iberiotoxine failed to prevent the LES relaxation caused nicorandil. Immunohistochemistry revealed that Kir6.1, Kir6.2, SUR1 and SUR2B subunit, which compose K^+ ATP channel, were expressed in rat lower esophagus. These findings suggest that nicorandil causes LES relaxation by activating K^+ ATP channel.
(COI: No)

P2-100

Force-inhibiting effect of phosphatase inhibitor on bovine ciliary muscle

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Purpose: Ciliary muscle is a smooth muscle with parasympathetic innervations and characterized by a rapid response to muscarinic receptor stimulation and sustained contraction. We recently reported that okadaic acid (OA), a phosphatase inhibitor, does not impaired carbachol (CCh)-induced contraction in bovine ciliary muscle (BCM). In order to address the regulatory mechanisms of ciliary muscle contraction, we examined the effects of selective PP2A inhibitors on BCM and guinea pig taenia cecum.
Methods: Smooth muscle strips were excised from bovine ciliary body and guinea pig taenia cecum. Muscle strips were contracted with CCh or ionomycin, and isometric tension was recorded. Various concentrations of OA, Fostriecin (Fos) and Rubratoxin A (RubA) were administered to contracted muscle strips.
Results: In CCh-induced contraction, low concentration of OA and Fos caused relaxation in taenia cecum, but not in BCM. RubA impaired contraction both in taenia cecum and BCM. On the other hand, in ionomycin-induced contraction, all three PP2A inhibitors impaired contraction both in taenia cecum and BCM.
Conclusion: These results strongly support the hypothesis that the force inhibiting effect of OA is due to PP2A inhibition but not non-specific activity. Since OA and Fos, but not RubA, failed to inhibit CCh-induced contraction in BCM, CCh may inhibit PP1 more potently in BCM than in any other smooth muscles.
(COI: No)

P2-101

Nucleotides dependence on the accerelating effects of myosin II inihibitors on the smooth muscle relaxation

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Blebbistatin, a myosin II inhibitor, suppress force development of skinned taenia cecum strips from guinea pig at any given Ca^{2+} concentration but had little effects on the phosphorylation of myosin regulatory light chain (Watanabe et al. Am J Physiol Cell Physiol 2010; 298:1118-1126). Also blebbistatin accelerates relaxation by removing Ca^{2+} from contracting preparations (Watanabe, J Physiol Sci 62:S160, 2012). These results suggest that blebbistatin suppressed skinned smooth muscle contraction through disturbing function and/or conformation of myosin heavy chain by the agent. Analyzing kinetics of the relaxation time courses of the skinned taenia cecum indicated that a portion of fast detaching cross-bridges to transfer to latch-bridges dissociating very slowly, and that, 1) blebbistatin suppressed transferring from fast detaching- cross bridges to slow detaching (latch)-bridges, and also 2) blebbistatin accelerated dissociation of the latch-bridges. To explore mechanisms of accelerating effects of blebbistatin on the skinned smooth muscle relaxation in detail, we investigated blebbistatin effects on the relaxation of the skinned taenia cecum in the presence of various nucleotides. In the absence of ATP, blebbistatin did not affect the relaxation process even in the presence of ADP. On the other hand, in the ATP containing solutions, blebbistatin accelerated the relaxation irrespective of ADP. The results suggest that blebbistatin affects conformational changes of myosin from ATP binding to ADP binding states, resulting in acceleration of dissociation of myosin from actin.
(COI: No)

P2-102

A new method for isolation of bovine ciliary muscle cells using Percoll gradient centrifugation

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In bovine ciliary muscle (BCM), stimulation of M_3-muscarinic receptors (M_3R) opens two types of non-selective cation channel with different unitary conductances (35 pS and 100 fS) which serve as major pathways for Ca^{2+} entry during sustained contraction. The molecular entities of these channels are still unknown, mainly because of the technical difficulty of obtaining BCM cells with sufficient purity. We describe here a new method by which one can obtain BCM cells with unprecedented quality and amount.
Methods The ciliary body dissected from bovine eye were treated with collagenase, and the dispersed cells were subjected centrifugation through discontinuous Percoll density-gradient of 1.050 and 1.060 g/mL. Cells were then collected from the 1.050/1.060 interface and cultured for 1-3 days before use. The intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) was monitored using the Fluo-4 fluorophore. Existence and localization of proteins were examined by immunofluorescence microscopy.
Results and discussion In the cultured BCM cell preparations, carbachol (2 μM) applied to the bath evoked a phasic and tonic increase of $[Ca^{2+}]_i$. Caffeine (20 mM) caused a phasic $[Ca^{2+}]_i$ elevation in the absence of extracellular Ca^{2+}. These responses were clearly observed in most cells, which were well stained with antibody against α-smooth muscle actin. Immunostaining also confirmed abundant expression of M_3R and STIM1 in the plasma and endoplasmic membranes. The present method allows us to obtain as many as 5×10^6 BCM cells with clear agonist sensitivity by a single-step centrifugation procedure.
(COI: No)

P2-103

Discovery of novel Salacia-derived components which specifically inhibit the ROK-mediated Ca^{2+}-sensitization of vascular smooth muscle contraction

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Rho-kinase-mediated Ca^{2+}-sensitization of vascular smooth muscle (VSM) contraction contributes to vasospasm. As upstream mediator for such pathological pathway, we identified sphingosylphosphorylcholine (SPC), which induced vasospasm in vivo, and its levels were extremely elevated in vasospastic patients. Furthermore we found that eicosapentaenoic acid (EPA) selectively inhibited the ROK-mediated Ca^{2+}-sensitization of VSM contraction, and clinically prevented cerebral vasospasm. In this study we aimed to identify a substitute for EPA as functional food from plants, because EPA is a component of fish oil, which is readily affected by marine pollution and has unstable supply. Contractile properties were assessed by the effects on Ca^{2+}-dependent contraction and Ca^{2+}-sensitization, which was induced by high K+-depolarization and SPC, respectively. After extensive screening, we found that extracts of Salacia, a woody climbing plant widely distributed in Asia and South America, strongly inhibited the Ca^{2+}-sensitization and very weakly blocked Ca^{2+}-dependent contraction. Liquid chromatography revealed that water-soluble fraction markedly inhibited the Ca^{2+}-sensitization, without affecting physiological Ca^{2+}-dependent contraction, while other fractions strongly inhibited the both types of contractions. These results suggest that Salacia extracts contain protective and therapeutic components for vasospasm.

(COI: Properly Declared)

P2-104

High-frequency sarcomeric auto-oscillations induced by heating in living neonatal cardiomyocytes of rat

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Previously, we developed an experimental system for simultaneous nano-scale analysis of single sarcomere dynamics and Ca^{2+} changes via expression of AcGFP in Z-discs (Shintani et al., J. Gen. Physiol., 2014). Using this system, we found that elevating temperature up to 40℃ by IR laser irradiation immediately generated fast-paced beating and sarcomeric oscillations outpacing the normal beating coupled with Ca^{2+} in a cardiomyocyte. This sarcomeric oscillations occurred independent of Ca^{2+} transients. We named this phenomena Hyperthermal Sarcomeric Oscillations (HSOs). The HSOs frequencies were stable, whereas under the conditions the normal beating is blocked, the HSOs were gradually organized. Therefore the coexistence of normal beating may be needed to induce stable HSOs.

(COI: No)

P2-105

Functional characterization of calcium holes in heart cells

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Calcium ion (Ca^{2+}) is a versatile intracellular signaling molecule that regulates various cellular functions, such as contraction, secretion, and gene expression. Preceding studies of the author indicate the existence of "calcium holes" in heart cells, a Ca^{2+}-deficient intracellular nanodomain that develops on the sarcolemmal membrane in the proximity (about 100 nm diameter) of a plasma membrane Ca^{2+}-ATPase (PMCA) molecule. Although Ca^{2+}-extrusion by PMCA in heart cells have so far been considered negligible, experimental evidence show that PMCA maintains the local Ca^{2+}-level in a calcium hole substantially lower than the global level. The operation of PMCA creates an encapsulated intracellular Ca^{2+}-signaling nanodomain that is distinct from the bulk Ca^{2+}-environment. Here, the author provide further evidence for the functional characteristics of calcium holes in heart cells, using whole-cell clamp and $[Ca^{2+}]_i$-microfluorimetry techniques. Under physiological conditions, inhibition of PMCA enhanced the amplitude of CICR-induced Na/Ca exchange current, and extended the duration of the action potential, suggesting an involvement of calcium holes in the regulation of cardiac E-C coupling and excitability. Possible physiological roles of PMCA and calcium holes in heart cells are also discussed.

(COI: No)

P2-106

Imaging of sarcomere dynamics in rat neonatal cardiomyocytes expressing stress fiber-like structures

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In the present study, we investigated whether sarcomeric dynamics is influenced by the development of stress fiber-like structures, and observed sarcomeres of rat neonatal cardiomyocytes by time-lapse imaging. Ventricular myocytes were isolated from 1-day-old Wistar rats, and cultured on collagen-coated glass bottom dishes. Stress fiber-like structures developed when myocytes were cultured for three to six days in the presence of basic fibroblast growth factor (FGF) or the actomyosin inhibitor N-benzyl-p-toluenesulphonamide (BTS). The magnitude of sarcomeric contractions did not significantly change upon treatment with FGF-2 or BTS. But lengthening velocity of a small number of sarcomere in series was slower than normal numbers. We hereby conclude that in neonatal cardiomyocytes, 1) intracellular stress fiber-like structures develop, and 2) the stress fiber-like structures do not significantly alter sarcomere contraction, 3) the contractility of remained sarcomeres is maintained.

(COI: No)

P2-107

Multipotent differentiation of human skeletal muscle-derived cells (Sk-Cs): Comparison to mouse Sk-Cs

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The differentiation potential of human Sk-Cs was examined, and compared to that of the mouse Sk-Cs. The samples (5-10g) were obtained from abdominal, and several leg muscles of 36 patients (17-79 years-old) undergoing prostate cancer, and leg amputation surgery following the accidents. All patients gave their informed consent to the aim and procedure. The Sk-Cs were isolated by originally conditioned collagenase solution, then, sorted as CD34-/CD45-/CD29+ (Sk-DN/29+) and CD34+/CD45- (Sk-34) cells, similar to the mouse case. The differentiation potentials were examined by cell culture and in vivo transplantation into the severely damaged muscles of athymic nude mice/rats. Interestingly, these two cell fractions could be clearly divided into highly myogenic (Sk-DN/29+) and multipotent stem cell (Sk-34) fractions as different from the mouse case. At 6 weeks of after separate transplantation of both cells, the former dominantly showed an active contribution to the muscle fiber regeneration, but the latter showed vigorous engraftment to the interstitium associate with the differentiation into Schwann cells, perineurial/endoneurial cells, and vascular endothelial cells and pericytes, as wholly corresponded to the previous mouse cases. Therefore, it was suggested that the human Sk-Cs was potentially applicable to the therapeutic autografts, expecting their multiple differentiation potential in vivo.

(COI: No)

P2-108

Enhancement of myosin heavy chain class I (MHC I) mRNA expression in C2C12 myocyte by multivalent cations

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Calcineurin is a protein phosphatase known as calcium-dependent serine-threonine phosphatase. We have previous reported that La^{3+} in the culture medium upregulated mRNA expression of myosin heavy chain class I (MHC I) and skeletal muscle modulators including interleukin-6 (IL-6) and heat shock protein 70 (HSP70) through the activation of calcineurin without increment of intracellular Ca^{2+}. In the present study we examined the effects of other multivalent cations, such as Gd^{3+} and Ni^{2+}, on expression of MHC, IL-6, and HSP70 mRNAs in C2C12 cells using real-time RT-PCR method. C2C12 cells were induced to differentiate to myotubes by medium exchange to D-MEM containing 2%FBS. The cells were incubated in D-MEM containing 2%FBS with multivalent cations, with or without cyclosporine A at the beginning of differentiation and removed after 24hr, and were maintained in differentiation medium. Our results are as follows: (1) The MHC I, IL-6, and HSP70 mRNA expressions were significantly increased by La^{3+}, but were decreased by cyclosporine A with or without La^{3+}. (2) The MHC I mRNA expression was significantly increased by the application of Gd^{3+} or Ni^{2+}, although the IL-6 and HSP70 mRNA expressions were not significantly upregulated by these cations. These results indicate that multivalent cations flowing into the cytosol may upregulate MHC I mRNA in calcineurin-dependent manner.

(COI: No)

P2-109

Spin-spin relaxation of 1H NMR signals from myosin filaments suspension with or without ATP

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The dynamic changes of water molecules structure surrounding contractile proteins might play an important role in cross-bridge cycling during contraction. The spin-spin relaxation process of 1H-NMR signals from suspension of myosin filaments prepared from rabbit could be well represented by the summation of several exponentials indicating that water molecules in the suspension could be conveniently grouped into several components based on the relaxation time constant (T2). The slowest two components (T2 around 0.4s and 0.15s) dominated over faster relaxation components. This may suggest that the potential of the water molecules existing around myosin filaments is high.

(COI: No)

P2-110

Analysis of novel protein in striated muscles that transcribe from the contiguous region of connectin gene

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Connectin is the largest protein that connects between Z-line and M-line of the sarcomere and functions as a molecular spring of vertebrate striated muscles. At the contiguous region of connectin gene on mammalian genomes, there is a gene for protein that function remain unknown. We found that this gene is expressed in various tissues including heart and skeletal muscles by RT-PCR experiments, and multiple splicing isoforms are produced from this gene. We also found that the protein (about 150kDa) from this gene is existed in heart and skeletal muscles by western blot test using newly produced antibody, and localized in the intercalated disk and the Z-line of sarcomere in heart muscle and the M-line of the sarcomere in skeletal muscles. The Z-line localization also confirmed by transfection of GFP-fusion 150kDa protein into muscle tissues and cultured muscle cells. To know the functions of the 150kDa isoforms in sarcomere genesis, we are now investigating the overexpression and restriction effects of them in cultured skeletal muscles.

(COI: No)

P2-111

Insulin-Growth Factor I Affects the Expression of Irisin

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Objective: Irisin was discovered from skeletal muscles and acts as a hormone to cause heat production by fat combustion. Therefore, irisin is considered to work similarly to the brown adipose tissue. In this study, we analyzed theeffect of insulin-like growth factor I (IGF-1) transferred to cultured muscle cells on the production of irisin.
Methods: IGF-1 gene was transferred into C2C12 cells, a mouse skeletal myoblastic cell line, by an electroporation method. Control (normal) and IGF-I-transferred CeC12 cells were cultured 1.0×10^5 cells / well. We observed every 12 hours until 48 hours. After examining the expression of fibronectin type III domain containing 5 (fndc5) gene at the molecular level by Lightcycler, we assessed potential correlations of irisin. In addition, we also measured isoform myosin heavy chain (MyHC), is the protein of muscle contraction were searched for association with irisin and MyHC.
Results & Discussion: The mRNA of fndc5 in the transferred group was expressed at 12 hours, but it was not expressed thereafter. MyHC-2d (flexible type), in the transferred group, was highly expressed at 12 hours. Therefore, we were considered that the transgenic IGF-1 changed to MyHC-2d by the surrounding environment. In addition, it was suggested that irisin was expressed in order to maintain the homeostasis.

(COI: No)

P2-112

Molecular genetics of type IV intermediate filament, synemin

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Intermediate filaments (IFs) are one of major components of the mammalian cytoskeleton. Synemin, a type IV IF protein, is known to form filaments with desmin and reinforce connection of Z disks and sarcolemmal proteins in skeletal muscle. But there is little knowledge about physiological functions of synemin. For investigating the functions of synemin in vivo, we generated synemin knockout (KO) mice. By observation of haematoxylin-eosin stain of several tissues, we could not find significant difference between wild type (WT) and KO mice. Furthermore we observed the relevance of synemin in muscular structure by immunofluorescence of myofibril markers, but there is no significant morphological change. After treadmill exercise, we observed muscle damage of WT and KO mice by measurement of the serum creatine phosphokinase level and staining of Evans blue, but there is not a significant difference. These results suggest synemin is not required for muscle development and maintenance of muscular structure.

(COI: No)

P2-113

Effects of disease-associated mutations in the central region on the RyR1 channels

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Type 1 ryanodine receptor (RyR1) is a Ca^{2+} release channel in the sarcoplasmic reticulum and the major target for muscle diseases, e.g., malignant hyperthermia (MH) and central core disease (CCD). It is widely believed that MH and CCD mutations cause hyperactivation of the Ca^{2+}-induced Ca^{2+} release (CICR), resulting in abnormal Ca^{2+} homeostasis in skeletal muscle. However, it remains unclear how the disease-associated mutations affect CICR. We have recently characterized several disease-associated mutations in the amino-terminal region by live-cell Ca^{2+} imaging and [^{3}H]ryanodine binding and found that these mutations divergently affect the gain (i.e., peak activity) and the sensitivity to activating Ca^{2+} of CICR. In this study, we extended this approach to 15 MH and MH/CCD mutations in the central region (1592-2508). The disease-associated mutations increased the gain and the sensitivity to activating Ca^{2+} in a site-dependent manner. The calculated CICR activity strongly correlated with the ER Ca^{2+} level, an index of Ca^{2+} leak. Importantly, the accelerated sensitivity to activating Ca^{2+} was linked to pathogenesis of CCD. Overall, the effects were similar to those of the amino-terminal mutations. The underlying molecular mechanism will be discussed.

(COI: No)

P2-114

Muscle glycogen fails to affect ryanodine receptor function and myofibrillar Ca^{2+} sensitivity in rat fast-twitch muscles

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Although the mechanisms by which decreases in muscle glycogen lead to muscle fatigue are not as well understood, some of previous studies have suggested that muscle fatigue induced by decreased glycogen is mediated through excitation-contraction uncoupling. The purpose of this study was to examine whether muscle glycogen depression causes impaired function of ryanodine receptor (RyR), a Ca^{2+} channel of sarcoplasmic reticulum, and/or reduced myofibrillar Ca^{2+} sensitivity. Wistar rats were randomly assigned to exercise or control groups. The rats in exercise group run on the rodent treadmill, then were subdivided into exercise-glycogen (EG) or exercise-fast (EF) groups and were allowed to rest for 2 h after exercise. During recovery, the EG rats were given 5% sucrose in water whereas the EF rats were given water only. Following recovery, the superficial regions of gastrocnemius muscles were excised and used for skinned fiber and biochemical experiments. The EG muscles exhibited a 1.5-hold higher glycogen concentration than the EF muscles. Skinned fiber and biochemical experiments indicated that there were no differences between the EF and EF muscles with regard to myofibrillar Ca^{2+} sensitivity and caffeine threshold of the RyR. These results suggest that changes in the muscle glycogen content may not affect the RyR and myofibrillar function.

(COI: No)

P2-115

Biomagnetic vector fields of gut functional syncytium

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Magnetic field detection of biological electric activity would provide a convenient estimate of the functional state of cellular organization, namely syncytium constructed with cell-to-cell electric coupling. In this study, we show the first real-time measurement of magnetic vector fields induced by biological propagating current in gut musculature, a typical functional syncytium, using an improved magnetoimpedance (MI) gradio-sensor with an amorphous metal wire core and a pair of detector coils. Biomagnetic waves of up to several nT were recorded in the magneto sensor placed ~1 mm below the sample under control conditions. The direction of magnetic waves altered depending on the rotation of the muscle layer and magneto sensor, indicating the existence of propagating intercellular currents. Tetraethyl ammonium (TEA) facilitated and nifedipine suppressed magnetic waves reflecting electric activity in smooth muscle, respectively, suggesting that L-type Ca2+ channels are responsible for the propagating current. The magnitude of magnetic waves rapidly decreased to ~30% by the initial and subsequent 1 mm separations between sample and sensor. The large distance effect is attributed to the feature of bioelectric circuits constructed by two reverse currents, i.e. intercellular propagating current and extracellular return current, separated by a small distance. We anticipate that the amorphous metal-based magneto sensor technology would make biomagentic fields a more realistic aspect in our lives, because these sensors are operated at ambient temperature without a magnetic shield.

(COI: No)

P2-116

Hydrogen sulfide inhibits motor activity of esophageal striated muscle in rats

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Inhibitory effects of hydrogen sulfide (H_2S) on the smooth muscle motility of the ileum and the colon have been reported. However, it is unclear whether H_2S can affect the esophageal motility. Therefore, the aim of the present study was to clarify the effects of H_2S on the motility of the esophageal striated muscle in the rat. An isolated segment of the rat esophagus was placed in an organ bath and the mechanical responses were recorded using a force transducer. Electrical stimulation of the vagus nerve evoked the contractile response in the esophageal segment. The vagally mediated contraction was inhibited by application of a H_2S donor, NaHS. NaHS did not affect the contraction induced by electrical field stimulation, which directly can excite the striated muscle not via vagus nerves. This shows that H_2S can influence not directly the striated muscle but neurons. RT-PCR revealed the expression of CBS and CSE mRNA in the esophageal tissue. These findings suggest that H_2S might be produced in the esophageal tissue and might regulate the motor activity of the esophageal striated muscle.

(COI: No)

P2-117

A genome-wide screen for genes involved in Helicobacter pylori-induced gastric carcinogenesis

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Helicobacter pylori (H. pylori) is strongly associated with atrophic gastritis, peptic ulcer, and gastric cancer. Its major oncoprotein CagA (Cytotoxin-associated gene A) translocates into gastric epithelial cells and disrupts host cell polarity and deregulates cell signaling, such as Receptor Tyrosine Kinase signaling and Wnt signaling. However, the molecular mechanism in which CagA develops gastric cancer is not fully understood. In this study we developed a transgenic Drosophila model in which CagA expression in the larval and adult eye induces eye defects. From a genome-wide overexpression screen of about 7,000 GS lines (from Drosophila Gene Search Project), we identified 30 genes whose forced expression strongly suppressed the CagA-induced rough eye phenotype. Some of these genes were found to be related to Ras-MAPK (Mitogen-activated Protein Kinase) signaling, Wnt signaling and signaling implicated in the establishment and maintenance of cellular polarity, which validates the findings of this screening. Of particular interest is that some gene products have been shown to function in gastric mucus secretion, which could suggest that the mucus secretion pathway may be inhibited by CagA. We will present some findings on the molecular mechanism of the inhibition.

(COI: No)

P2-118

Functional analyses of dipeptidase-1 (DPEP1) using a colon/gastric cancer-derived cell line, HCC56

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DPEP1, a glycosylphosphatidylinositol (GPI)-anchored protein, is highly expressed in colon cancer and thus proposed as a prognostic marker, but its function has yet to be elucidated. We found that HCC56 cells, a colon/gastric cancer cell line, expressed DPEP1 much higher than other colon cancer-derived cell lines, such as LoVo, RKO, HT29, SW480, CaCO2. In colonies of HCC56 cells, immunofluorescence signal for DPEP1 was often observed along the cell surface as dot appearance, and was well colocalized with another GPI-anchored protein, CD59. DPEP1-knockdown in HCC56 cells did not affect cellular activities of proliferation in both culture and xenograft models. However, DNA damage after the oxidative stress induced by disodium hydrogen arsenate heptahydrate was more severe in the DPEP1-knochdown cells than in control cells. These results suggest that DPEP1 expression confer tolerance against oxidative stress probably by its ability to cleavage cysteinylglycine, a glutathione metabolite.

(COI: No)

P2-119

Morphological and functional studies on the gastrointestinal tract in a mouse model of chronic renal failure

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Introduction: It has been reported that mice with 5/6 nephrectomy-induced chronic renal failure (CRF) have reduced gastrointestinal transit (GIT) and increased fecal moisture content (FMC). We have recently shown that feeding adenine (0.2%, w/w) to mice can be used as a model of CRF. Here, we investigated the possible effects of adenine-induced CRF on the GIT physiology and histology in mice.
Methods: The effects of CRF induced by feeding adenine (0.2%, w/w for 2 or 4 weeks) on the gastric emptying index (GEI), GIT, FMC and bead expulsion test (BET) were investigated.
Results: Feeding adenine for 2 or 4 weeks resulted in CRF. The BET was significantly increased in mice given adenine for 2 but not 4 weeks, while the GEI was significantly increased in mice treated with adenine for 4 but not 2 weeks. No significant differences between control and adenine-treated mice were found in GIT, FMC or the histology of the different parts of the gut. Acetylcholine-induced contractions of the ileum of adenine-treated rats were not significantly different from those of the controls.
Conclusion: Feeding adenine for either 2 or 4 weeks resulted in CRF, but it would appear that this model produces effects on the gastrointestinal tract that are milder than those reported before in animal models with 5/6 nephrectomy-induced CRF.

(COI: No)

P2-120

Lectin-based histochemical mapping of fucosylated glycoproteins in mouse intestinal tract

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Recently, clarification of the biological roles of protein glycosylation is receiving special attention. A glycan moiety of glycoproteins is found to be functionally linked to various biological processes through modulating protein functions, such as signal transduction and molecular interactions. Thus, characterization of proteins carrying an interested glycan is a key to clarify the role of the glycan.
Spatiotemporal distributions of glycans have been investigated by lectin-histochemistry in various organs, tissues and cells. However, histochemical profiles of their carrier proteins are hardly revealed. This is because of difficulty in identification of the carrier proteins and specific detection of a particular protein carrying an interested glycan. To overcome these issues, we utilized an approach integrating glycoproteomic analysis for identification of the carrier protein and in situ Proximity Ligation Assay for histochemical detection of the targeted glycoprotein. Here, we report our recent achievements in the mapping of fucosylated glycoproteins in mouse intestinal tract. Fucosylated glycans expressed by the intestinal epithelial cells are reported to play pivotal roles in maintaining intestinal homeostasis. Based on the protein identification result obtained by glycoproteomic analysis, distribution of a particular fucosylated glycoprotein was examined. Our approach will facilitate unraveling the roles of the intestinal fucosylated glycans.

(COI: No)

P2-121

Vitamin A status in a short bowel rat model

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Short bowel (SB) syndrome causes the malabsorption of various nutrients. Among them, vitamin A is important for many physiological activities. Vitamin A is taken up by absorptive epithelial cells of the small intestine and discharged into lymphatics as a component of chylomicrons and delivered to the liver. We used a rat model of SB syndrome to assess its effects on the expression of genes associated with the absorption, transport and metabolism of vitamin A. In SB animals, the small bowel was resected from a point five cm distal to the ligament of Treitz to a point ten cm proximal to the ileocecal junction, resulting in a 75% resection of the small intestine. In SB rats, intestinal expression levels of mRNAs for cellular retinol-binding protein II (CRBP II, gene symbol Rbp2) and apolipoprotein A-IV (gene symbol Apoa4) were higher than in shams. In SB rats, the ileal retinol content and the jejunal retinyl esters content were lower than in sham rats. These results suggest that the elevated expression levels of Rbp2 and Apoa4 mRNAs in SB rats contribute to the effective esterification and transport of vitamin A.

(COI: No)

P2-122

A mathematical model of glucose absorption in small intestinal epithelium

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It is generally accepted that glucose absorption in small intestine is mediated by Na^+-dependent glucose cotransporter (SGLT) in the apical membrane and glucose transporter (GLUT) in the basolateral membrane. In the present study, we have tried to construct a mathematical model of glucose transport by small intestinal epithelial cell using MATLAB/Simulink. The apical membrane contained $2Na^+$-1glucose cotransporter (SGLT), Cl^- and HCO_3^- conductances, $1Cl^-$-$1HCO_3^-$ exchanger (AE), and Na^+-H^+ exchanger (NHE). The basolateral membrane contained glucose permeability (GLUT), Na^+-K^+ pump, K^+ conductance, Na^+-K^+-$2Cl^-$ cotransporter, AE, and NHE. Both apical and basolateral membranes have CO_2 and H_2O permeabilities. The paracellular pathway contained Na^+, K^+, and Cl^- conductances. The permeability values of those ion channels/transporters/pump were optimized to reproduce reasonable values for intracellular parameters (pH 7.2, Cl^- 40 mM, Na^+ 10 mM, basolateral membrane potential -60 mV) and the published experimental data of glucose transport rate (Hardin *et al*, Gut. 2000). In the constructed model, when luminal glucose was elevated from 5 to 10 mM (basolateral glucose was kept at 5 mM), intracellular Na^+ increased from 9.7 to 13.7 mM, intracellular glucose increased from 5.01 to 5.04 mM, and basolateral membrane was depolarized from -67.3 to -59.7 mV, while the rate of glucose absorption increased by ~5 times from 2.5 to 12.3 nmol cm^{-2} min^{-1}. The apparent K_m for luminal glucose was 26.9 mM. Our mathematical successfully reproduced glucose absorption in small intestinal epithelium.

(COI: No)

P2-123

Morphological changes in tunica muscularis in adenoma regions of the small intestine in *Apc*$^{Min/+}$ mice, with special reference to the interstitial cells of Cajal

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Apc$^{Min/+}$ mouse, a mouse model for familial adenomatous polyposis, spontaneously develops numerous adenomas in the small intestine. We noted that the thickening of tunica muscularis and the increase of interstitial cells of Cajal (ICC) occurred in the adenoma region of *Apc*$^{Min/+}$ mice. ICC have been widely acknowledged as being essential for the normal function of the digestive tract, acting both as pacemaker cells and as mediators between nerves and smooth muscle cells. The present study has been designed to clarify the morphological changes in tunica muscularis in the small intestinal adenoma regions together with the morphological characteristics of ICC. Male C57BL/6J-*Apc*$^{Min/+}$ mice aged 7 months were used. Short segments including adenoma regions were observed by the immunohistochemistry and electron microscopy. In the adenoma region, the increase in the mass of intercellular substances caused the thickening of the tunica muscularis, resulting in loss of attachments among smooth muscle cells. Dense distribution of ICC was observed to associate with the myenteric plexus (ICC-MP). Cytoplasmic processes of ICC-MP elongated into circular and longitudinal muscle layers. These processes were closely associated with the nerves and showed the gap-junction protein expression. These results may suggest that ICC compensate the lack of connections among smooth muscle cells with their mediator function.

(COI: No)

P2-124

Expression of muscarinic acetylcholine receptors on ICC and fibroblast-like cells of the mouse gastrointestinal tract

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Acetylcholine is the major neurotransmitter that induces gastrointestinal (GI) smooth muscle contractions. Cholinergic innervation is mediated by M2 and M3 muscarinic acetylcholine receptors expressed on the smooth muscle cells. In addition, we have reported that the interstitial cells of Cajal (ICC), regulatory cells of the GI motility, also express M2 receptor. In the musculature of GI tract, another type of interstitial cells called fibroblast-like cells (FLC) exists. Recent studies have suggested that FLC also contribute to mediate the neurotransmission to smooth muscle cells. In the present study, we examined the expression of M2 and M3 receptors on ICC and FLC. We first isolated these cells from the muscle layer of the small intestine by FACS sorting using KIT-GFP (for ICC) and PDGFR α-GFP (for FLC) mice. The ratios of ICC and FLC to total cells of the muscle layer were 1% and 4-5%, respectively. Quantitative RT-PCR analysis showed that M2 and M3 mRNA were highly expressed in isolated ICC and FLC, whereas the expression of M1, M4 and M5 were not detected in both cell types. By immunohistochemistry, M2 receptor immunoreactivity was detected in ICC and FLC. These M2 receptor-immunoreactive cells were associated with cholinergic nerve bundles. In addition, by using analysis of microarray data, isolated ICC and FLC highly expressed inositol transporters related with M2 receptor. These results suggested that FLC as well as ICC expressed muscarinic acetylcholine receptors and were responsible for cholinergic neurotransmission in the muscle layer of GI tract.

(COI: No)

P2-125

Regional differences in the structure of rat intestinal villi

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The structural differences between the rat duodenum, jejunum, and ileum were investigated by scanning electron microscopy (SEM) and light microscopy (LM). Male Wistar rats, eight to thirteen weeks of age, were used. The animals were fixed by perfusion of 4% paraformaldehyde, followed by the removal of the duodenum, jejunum and ileum. Some of these tissues were further immersed in 2% glutaraldehyde and prepared for SEM, while some others were used for LM. The shape of rat intestinal villi was leaf-like, but they tended to be rather thick in the duodenum. The height of villi was about 460 μm in the jejunum and about 290 μm in the ileum. The width was about 70 μm in the jejunum and 50 μm in the ileum. Paneth cells were observed mainly in the ileum. Filamentous microorganisms (presumably bacilli) were often embedded in iliac villi, but not found in the starved rat. Further structural details will be discussed especially on the shape and arrangement of lymphatics and blood vessels.

(COI: No)

P2-126

Tenascin C producing cells in the gastrointestinal tract of adult mice

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Tenascin C (TnC) is an extracellular matrix (ECM) protein that is expressed during embryogenesis, wound healing and tumorigenesis. TnC promotes the de-adhesion of cells to ECM, and up-regulate cell migration and proliferation. On the other hand, little is known about the expression of TnC in healthy adult tissues. We reported the expression of TnC in the gastrointestinal (GI) tract of adult mice at the last annual meeting. We have revealed organ-specific TnC expression in the GI tract. In the present study we identified the TnC producing cells in the normal adult GI tract using TnC-lacZ transgenic mice. TnC producing cells were detected by immunohistochemistry using anti-β-gal antibody. TnC molecules were widely distributed in the extracellular space of mucosa and muscle layer throughout the GI tract. Fibroblast marker and β-Gal double-positive cells were observed in the lamina propria and within the muscle layer. Smooth muscle cells of the lamina muscularis mucosae in stomach, and longitudinal muscle layers in stomach and colon show dense β-Gal immunoreactivities (IR). In addition, moderate β-Gal IR were observed in smooth muscle cells in circular muscle layers throughout the GI tract. No co-localization of β-Gal and neural cell marker was observed. Some KIT immunopositive ICC within the myenteric plexus region seemed to possess β-Gal IR. In conclusion, fibroblast, smooth muscle and ICC produce TnC. TnC can effects the cell adhesion property of the connective tissue, so they may produce organ-specific micro-environment in the GI tract.

(COI: No)

P2-127

Studies on neurogenesis of enteric neurons in *c-kit* mutant mouse after benzalkonium chloride-induced neuron injury

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Interstitial cells of Cajal (ICC) are mesenchymal cells localized along the gastrointestinal tract and they have close interactions with enteric nervous system (ENS) both morphologically and functionally. To reveal an implication of ICC in ENS regeneration, we used ileum of *c-kit* mutant mice (WBB6F1/Kit-*Kit*$^{W/Kit^{W-v}}$/Slc: *W/W*^{v}), which are known as ICC deficient mice. After ENS injury by benzalkonium chloride (BAC), the neurons in myenteric plexus (MP) were disappeared both in wild type (C57BL/6NcrSlc: WT) and *W/W*^{v} mutant, however other structures such as mucosal epithelium and smooth muscle remained as normal. Two weeks after the injury, the recovery of MP was not observed in neither WT nor *W/W*^{v}, however the elongation of nerve fibers along the longitudinal muscle layer were detected in both animals. In addition, especially in *W/W*^{v} mice, ectopic NADPH positive cells were observed in the longitudinal muscle layer or the subserosal layer, where usually enteric neurons were not observed. These cells were also labeled by PGP9.5 antibody, and therefore these were considered as neurons. Although neurogenesis is hardly observed under normal condition of wild type intestine, the apparent ENS neurogenesis is seen in ileum of *W/W*^{v}. These results may suggest that a deletion of ICC or some factors associated with *c-kit* are contributed to the ENS neurogenesis after injury.

(COI: No)

P2-128

Immunohistochemical study of a Membrane Skeletal Protein, Membrane Protein Palmitoylated 6 (MPP6), in Mouse Small Intestine

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Membrane protein palmitoylated 1 (MPP1) is a membrane skeletal protein that interacts with a 4.1 family protein, 4.1R, in erythrocytes. We already identified another MPP family MPP6 interaction with 4.1G in the mouse peripheral nervous system. In this study, we examined immunolocalizations of MPP6 in mouse small intestines, and compared them with those of 4.1B that we had already reported in intestinal epithelial cells. Cryosections or paraffin sections of small intestines of wild-type or 4.1B-deficient mice were immunostained for MPP6, 4.1B, and E-cadherin. In the small intestines, molecular weight of MPP6 was about 60kD with Western blot, and it was immunostained at lateral portions of all epithelial cells from crypts to intestinal villi, as well as in Auerbach's plexus probably reflecting enteric glia. In the epithelial cells, immunostained areas of MPP6 were slightly different from those of 4.1B whose immunolocalization was restricted in the intestinal villi. The immunostaining pattern of E-cadherin in epithelial cells was similar to that of MPP6. The MPP6 immunolocalization in small intestinal epithelial cells of the 4.1B-deficient mouse was similar to that of the wild-type mouse. Thus, we demonstrated the MPP6 immunolocalizations in mouse small intestines, suggesting that 4.1B in the intestinal epithelial cells was not essential for the MPP6 sorting.

(COI: No)

P2-129

Visualization of the entire differentiation process of murine M cells: suppression of their maturation in cecal patches

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The microfold (M) cell residing in the follicle-associated epithelium (FAE) is a specialized epithelial cell that initiates mucosal immune responses by sampling luminal antigens. The differentiation process of M cells remains unclear due to limitations of analytical methods. Here we found that M cells were classified into two functionally different subtypes based on the expression of Glycoprotein 2 (GP2) by newly developed image cytometric analysis. GP2-high M cells actively took up luminal microbeads whereas GP2-negative or low cells scarcely ingested them, even though both subsets equally expressed the other M-cell signature genes, suggesting that GP2-high M cells represent functionally mature M cells. Further, the GP2-high mature M cells were abundant in Peyer's patch but sparse in the cecal patch: this was most likely due to a decrease in the nuclear translocation of RelB, a downstream transcription factor for the receptor activator of NF-κB signaling. Given that murine cecum contains a protrusion of beneficial commensals, the restriction of M-cell activity might contribute to preventing the onset of any excessive immune response to the commensals through decelerating the M-cell-dependent uptake of microorganisms.

(COI: No)

P2-130

Short-chain fatty acid activates bicarbonate absorption or proton secretion in rat rectal colon

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Short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate are synthesized from dietary carbohydrate by colonic bacteria fermentation. These SCFAs are considered to contribute not only to an energy source or prevention of cancer but also in regulating ion transport. However, the SCFA's effects on ion transport and intracellular environment remain unknown. In this study, I showed the effects of a 30 mM butyrate application on rat colon with short-circuit current (I_{SC}) measurements. Butyrate application shifted I_{SC} toward negative direction. Because electroneutral monocarboxylate transporters (MCT1 and/or MCT4) co-transports proton with SCFAs including butyrate, it could be considered that this I_{SC} shift was occurred by electrogenic HCO_3^- absorption or H^+ secretion in order to neutralize intracellular environment. In rat colon, there are some channels or transporters which concern with HCO_3^- transport, but only cAMP-activated Cl^- channel, cystic fibrosis transmembrane conductance regulator (CFTR), matched to the direction both HCO_3^- transport and I_{SC} shift. In fact, CFTR inhibitor, CFTRinh172 reduced this I_{SC} shift induced by butyrate application. On the other hand, H^+ secretion on apical membrane also matched this I_{SC} shift to neutralize intracellular pH which was contributed by H^+-ATPase and/or H^+-K^+-ATPase in cooperation with K^+ channel. The aim of this study was to reveal those channel and/or transporters contribution on the condition of SCFA application.

(COI: No)

P2-131

Secretory effects of Xenin on rat colonic epithelia

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Xenin-25 (Xen) is a 25 amino acid neurotensin-related peptide produced by a GIP-producing K cells in the small intestine. In animals, Xen delays gastric emptying, increases gastric motility induces gall bladder contractions and reduces food intake. Many of these effects are known to be mediated by enteric neurons. Xen has multiple actions on gastrointestinal activity, however, there has been no report on the ion transport in the gut. In this study, we have investigated the effect of Xen on ion transport in rat colon. Xen was synthesized by a solid-phase methodology with Fmoc-strategy using an automated peptide synthesizer (Model Pioneer; Life Technologies, CA, USA). The crude peptide was purified by reverse-phase HPLC (Delta 600 HPLC system; Waters, MA, USA). The homogeneity of the purified peptide was confirmed by analytical HPLC, MALDI-TOF mass spectrometry, and amino acid analysis. Smooth muscle removed mucosa-submucosa preparations of rat large intestine were mounted on Ussing flux chambers and short-circuit current (Isc) was measured as an index of transepithelial ion transport. In addition, expression of Xen in the colon was analyzed by PCR. In Ussing chamber experiments, serosal application of Xen (10-9 ~ 10-6 M) concentration-dependently induced a transient increase in Isc in middle colon, distal colon and rectum but not in proximal colon. These results suggest that Xen functions as a mediator on the ion transport in the rat colon.

(COI: No)

P2-132

Kinase activity of TRPM7 regulates lipid metabolism

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Transient receptor potential melastatin 7(TRPM7) is a member of TRP family of cation channels involved in sensory pathways and respond to various environmental stimuli. TRPM7 is a unique fusion of an ion channel and a C-terminus kinase domain. However, the physiological functions of TRPM7 and its kinase activity in vivo remain largely unclear. We generated kinase-inactive mutant mice and analyzed their phenotype. TRPM7 mutant mice show normal ion channel activity without noticeable kinase function in cells isolated from adult animals. These mice have normal body weight, food intake and general locomotor activity. Screening of serum clinical parameters showed that serum Ca^{2+} and Mg^{2+} levels were not altered, but serum triglyceride and total cholesterol were significantly decreased. High-fat diet increased the accumulation of fat in the liver compared to wild type mice. Our findings define TRPM7 kinase activity as a key cell signaling component that regulates lipid metabolism in the liver.

(COI: No)

P2-133

Silencing of Delta-like3 expression by DNA methylation and histone modification in hepatocellular carcinoma cells

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Introduction: Development and progression of hepatocellular carcinoma is caused by a multistep mechanism. Activation of oncogenes and inactivation of tumor suppressor genes by genetic or epigenetic aberrance are involved in hepatocarcinogenesis. We previously reported that Delta-like 3(DLL3)gene, a member of DSL ligands for Notch receptor is aberrantly methylated and DLL3 expression induces cellular apoptosis in HCC cell line, HuH2. (Aim) The aim of this study is to investigate the epigenetic mechanism of DLL3 silencing in HCC.
Materials and Methods: 1. Immunohistochemical study of DLL3 in HCC tissues. 2. Immunohistochemical study of methylated histone H3 lysine 27(H3K27me3) in HCC tissues. 3. Reactivation assay of DLL3 expression with DNA methylation inhibitor (5-Aza-dC), histone deacethylase inhibitor (TSA), and histone methyltransferase inhibitor (DZNep) in HuH2 cells.
Results: 1. DLL3 expression is not observed in 50% (18/36) of the HCC cases whereas DLL3 is expressed in all (9/9) corresponding non-cancerous tissues. 2. H3K27me3 is observed in 60% of the HCC cases and 80% of DLL3-negative HCC cases. 3. DLL3 expression is reactivated by the treatment of 5-Aza-dC and TSA, whereas no effect is observed by the treatment of DZNep.
Discussion: DLL3 expression in regulated by DNA methylation and may influence hapatocarcinogenesis. However H3K27me3 did not affect DLL3 silencing, further experiments are now undergoing concerning the involvement of other histone modifications.

(COI: No)

P2-134

Effect of starvation on the expression of a lipid droplet protein, ADRP, in the mouse liver

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Adipose differentiation-related protein (ADRP) is a major protein associated with lipid droplet in various types of cells. In this study, we analyzed the effect of starvation on the expression of ADRP in the mouse liver. Adult male ddY mice were starved for 24 hours, and then the livers were collected for histological analysis. When the mRNA expression of ADRP was analyzed by *in situ* hybridization technique, it was significantly increased in the liver of the starved mice. Abundant number of lipid droplets was observed in the cytoplasm of hepatocytes in the starved mice, and an intense immunoreactivity for ADRP was found in the membrane of increased lipid droplets. When lipids contents were stained using BODIPY 493/503 or Sudan III on ADRP-immunostained sections, the lipid droplets surrounded by ADRP immunoreaction tended to be negative in reaction for BODIPY or Sudan III, in contrast to lipid droplets without ADRP immunoreactivity. Immuno-electron microscopic observation revealed the specific localization of ADRP on the edges of vacuoles which were not filled with lipid contents. Because the mRNA expression of adipose triglyceride lipase (ATGL), a late-limiting lipase, was significantly increased in the liver of starved mice, the ADRP-positive lipid droplets may be under a lipolytic condition.

(COI: No)

P2-135

Fibroblast growth factor-5 participates in the progression of hepatic fibrosis

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Non-alcoholic steatohepatitis (NASH) is characterized by the presence of steatosis, inflammation, and fibrosis and is believed to develop via a "two-hit process"; however, its pathophysiology remains unclear. Fibroblast growth factors (FGFs) are heparin-binding polypeptides with diverse biological activities in many developmental and metabolic processes. In particular, FGF5 is associated with high blood pressure. We investigated the function of FGF5 in vivo using spontaneously Fgf5 null mice and explored the role of diet in the development of NASH. Mice fed a high-fat diet gained little weight and had higher serum alanine transaminase, aspartate amino transferase, and non-high-density lipoprotein-cholesterol levels. Liver histology indicated marked inflammation, focal necrosis, fat deposition, and fibrosis, similar to the characteristics of NASH. FGF5 and a high-fat diet play significant roles in the pathophysiology of hepatic fibrosis and Fgf5 null mice may provide a suitable model for liver fibrosis or NASH.

(COI: No)

P2-136

Immunohistochemical localization of CD133, nestin, Bmi-1 and mTOR in the pancreas of rat models of type 1 and type 2 diabetes mellitus

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Stem cell-related markers (CD133, nestin and Bmi-1) and mammalian target of rapamycin (mTOR) were detected in the pancreas of rat models of type 1 and type 2 diabetes mellitus (DM). As a model of type 1 DM, Komeda diabetes-prone (KDP) rat and control rat (KND rat) were used. As a model of type 2 DM, spontaneously diabetic Torii (SDT) rat and control rat (SD rat) were used. In each control rat, pancreatic islets were strongly positive for CD133, nestin, Bmi-1 and mTOR. In the KDP rat, CD133, nestin, Bmi-1and mTOR positive cells did not show cellular mass like pancreatic islets. Theses positive cells were associated with invasion by mononuclear cells. In the SDT rat, CD133, nestin, Bmi-1 and mTOR positive cells also did not show cellular mass like pancreatic islets. These positive cells were associated with increased fibrillar element. The number of nestin positive cell was decreased. Present study suggests that nestin may play an important role in the maintenance of pancreatic islet, especially in the SDT rat.

Reference:
Murata E, Matsumoto S, Shuto M and Akita M. J Stem Cells Res, Rev & Rep. 2014; 1(1):6

(COI: No)

P2-137

Inhibitory effects of Saiko-keishi-to (TJ-10) on pancreatitis-reduced pain in a rat nodel of chronic pancreatitis

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Abdominal pain is one of the most important symptoms in chronic pancreatitis, presenting in 80-90% patients during the course of the disease. The aim of the present study was to investigate the role and underlying mechanisms of Saiko-keishi-to (TJ-10) in a rat model of chronic pancreatitis. In the present study, male Lew rats (150-160 g) were employed as induced-chronic pancreatitis (CP) model by injection of dibutyltin dichloride (DBTC) (8 mg/kg BW) into the tail vein. TJ-10 was given daily by mixed in feed at a dose of 10 g/kg of body weight, starting from two weeks after CP induction. The behavioral testing of mechanical response was tested using von Frey filaments. After treatment of TJ-10, rats were sacrificed, and pancreatic tissues, dorsal root ganglia (DRG) and thoracic spinal cord (T9-12) were harvested for investigating the expression of fibrosis/pain-related factors. Treatment of CP with TJ-10 decreased the histological lesion, and reduced the expression of TGF-β1, Smad2 and Smad3, improved the fibrosis and pancreatitis-induced pain. The $\alpha 2\delta$-1 precession of T9-12 in rats with chronic pancreatitis was declined. The present study suggested that repeated administration of TJ-10 daily could reduce mechanical hypersensitivity in the upper abdomen and produce an analgesic effect in a rat model of chronic pancreatitis. The down-regulation of $\alpha 2\delta$-1 calcium channel subunit might be one of the mechanisms underlying the analgesic effect of TJ-10.

(COI: No)

P2-138

Deletion of the tight junction protein claudin 15 causes malabsorption of ologopeptide in murine intestine

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It is known that the claudin family of tight junction proteins is critical in determining paracellular ionic permeability and selectivity. We have shown that loss of claudin 15 results in decreased luminal Na+ concentration and glucose malabsorption in the small intestine. To gain further insight into the relationship between intestinal Na+ metabolism and changes in peptide absorption induced by the loss of claudin 15, we investigated the site of absorption of electrolytes and peptide in claudin 15 knockout (cldn15KO) mice. Mice were fed a powdered diet supplemented with 14C-polyethylene glycol (PEG) 4000 as a non-absorbable marker and 3H-Gly-Sar (non-hydrolyzable dipeptide). Three hours after feeding, the small intestine was isolated and divided into six segments, the luminal contents collected for analysis of Na+, K+, and Cl- concentrations and the level of 14C-PEG4000. Na+, K+, and Cl- concentrations were determined using ion-selective electrodes. Gastric emptying time, assessed by measuring 14C-PEG4000, was decreased in cldn15KO compared to wild-type mice. Total luminal contents in the small intestine were increased in cldn15KO mice and the retention time of digesta in the upper jejunum was increased approximately 3-fold compared with wild-type mice. Robust Na+ secretion and rate of absorption were observed in the upper jejunum in wild-type mice and this was attenuated in cldn15KO mice. The rate of K+ absorption was increased in cldn15KO mice in the lower ileum. Total luminal Gly-Sar were increased, while absorption rates of Gly-Sar were decreased, in the upper jejunum of cldn15KO mice.

(COI: No)

P2-139

Forensic estimation of age at death using synchrotron-radiation micro-CT of human teeth

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Teeth are the hardest parts of the human body and they survive well under adverse conditions and are the basis for several methods for age-at-death estimation. One established method for the estimation of a person's age is based on the observation that dentinal tubules gradually fill with mineral (secondary dentine) over time. This infilling begins at the apex of the tooth root and proceeds towards the crown. The method of age estimation depends upon being able to establish a relationship between the level of secondary dentine formation and the chronological age of an individual. Synchrotron radiation micro-CT offers the possibility of low noise imaging without any beam hardening and has the potential to overcome the problems. In this study, we acquired micro-CT data from 50 single-rooted human teeth from individuals of known ages, detected the limit of secondary dentine formation and calculate fractional change in the volume of the tooth root occupied by this. For consistency of the image analysis, 17mandibular incisors(12 males and 5 females of 40-79 years old) without caries or other abnormalities were used. The ratio between pulp cavity and dentinal volume decreased with increasing age. The coefficient of determination by multiple regression analysis for 17 specimens was 0.459. Age estimation based one age-related changes in pulp cavity to dentinal volume ratio as well as three-dimentional reconstruction of tooth structure has been successful using synchrotoron- radiation micro-CT.

(COI: No)

P2-140

Regulatory mechanism in differentiation of mesenchymal cells during tooth development

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Objective: While previous studies have suggested some signaling molecules involved in tooth development, but mechanism of differentiation of mesenchymal cells remains unclear in detail. Here, we investigated the expression patterns of some signaling molecules which may be involving in formation of blood vessels during tooth development.
Method: Immunohistochemically we stained serial sections of dental tissues with the antibody against the molecules may be involving in formation of blood vessels. And also we performed in situ hybridization by using the probes of above-mentioned molecules.
Result: These molecules were observed in the mesenchymal layers, dental papilla and around the blood vessels in tooth germ. And also the localization of transcripts of these molecules were observed in mesenchymal cells in tooth germ.
Conclusion: In this research, we confirmed the molecules might have involved in formation of blood vessels during tooth development. *This work was supported by JSPS KAKENHI Grant Numbers 22592052, 26462800.

(COI: No)

P2-141

Immunohistochemical Localization of Bmi1 during odontoblast differentiation and regeneration

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Bmi1 is a polycomb protein localized in stem cells and regulates expression of differentiation genes. In this study, to analyze the role of Bmi1 during dentinogenesis, we examined the immunohistochemical localization of Bmi1 during rat tooth development as well as after cavity preparation. Bmi1 localization was hardly detected in dental mesenchyme at the bud and cap stages. After the bell stage, this protein became detectable, being localized in odontoblasts just beginning dentin matrix secretion and in preodontoblasts near these odontoblasts. As dentin formation progressed, Bmi1 immunoreactivity in the odontoblasts decreased in intensity. After cavity preparation, cells lining the dentin and some pulp cells were immunopositive for Bmi1 at 4 days. Odontoblast-like cells forming reparative dentin were immunopositive at 1 week, whereas this immunoreactivity disappeared after 8 weeks. Next, we further analyzed the function of Bmi1 using dental pulp cells in vitro. Following stimulation with BMP-2, Bmi1 expression was elevated. siRNA knockdown of Bmi1 in these cells reduced the expression of odonto- and osteo-blast differentiation marker genes such as Runx2, Osterix, and Osteocalcin. Taken together, these findings suggest that Bmi1 was localized to the odontoblast lineage cells in their early differentiation stages, and might positively regulate their differentiation.

(COI: No)

P2-142

IFT88 plays a role in the ciliogenesis even during mitosis

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IFT88 is known to be required for the ciliogenesis in most of the quiescent cells. We have previously reported that IFT88 regulate the odontoblastic differentiation through primary cilia. Moreover, conventional idea holds that IFT88 chiefly function in mitogenic events during mitosis. However, since we revealed that IFT88 in preodontobastic KN-3 cells functions on the ciliogenesis even during mitosis, we report here. When KN-3 cells were transfected with a retroviral expression vector for *Ift88* shRNA, cell adhesion, formation of lamellipodia, and cell proliferation were impaired. In addition, *Ift88* knockdown decreased the expression of molecular marker for mitosis. In this case, we reproduced the phenotype by inhibiting the mitosis, and obtained a similar phenotype to those inhibited by *Ift88* knockdown. This result suggested that IFT88 take part in the regulation of cell cycle. From another standpoint, while the ratio of cilia was not changed, the intensity of acetylated α-tubulin-positive protruding structures was decreased by *Ift88* knockdown even during mitosis. Moreover, the non-canonical Wnt signal, which is mediated by primary cilia, was suppressed. To recapitulate the phenomena, we inhibited the non-canonical Wnt signal by an inhibitor, and obtained a similar phenotype to those inhibited by *Ift88* knockdown. This result suggested that IFT88 functions on the ciliogenesis even during mitosis. Collectively, IFT88 exerts its effects on mitotic profiles of KN-3 cells not only through extraciliary pathway but also through ciliary pathway.

(COI: No)

P2-143

Effects of the thyroid hormone on tooth development in newts

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Objective: It has been suggested that the thyroid hormone receptor and thyroid hormone are contribute to the development of the teeth and the regeneration of alveolar bone following amputation in newts. However, the relationshipbetween the mRNA transcript levels of THRs in alveolar bone is unknown in blocking the effect of thyroid hormone.
Method: Propylthiouracil (PTU) is a thiouracil-derived drug used to blocking the effect of thyroid hormone. Japanese newts (*Cynops pyrrhogaster*) were maintained in aqueous solutions with or without 100 μg/ml PTU for one month and tracked the sequential development of the tooth cap, the tooth bud, and ultimately the maturation of the sequential tooth germ. Fifty days after the amputation procedure of the right mandible alveolar bone, the newts were observed the effect of PTU by the expression levels of mRNA of thyroid hormone receptor detected in-situ hybridization and real time RT-PCR.
Result: The amputated mandible sites were observed microscopically. The level of mineralized area of alveolar bone in amputation with PTU treatment was significantly smaller than that in the amputation without PTU treatment (Student's t-test, $p<0.001$).
Conclusion: These data indicate that PTU may affect the tooth germ development and the regeneration of alveolar bone in newts.

(COI: No)

P2-144

Histology and elemental composition of the cervical enamel in human unworn mesiodenses

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Objectives: The purpose of this study is to clear the histological structure and elemental composition of the cervical enamel in human unworn mesiodenses.
Methods: Re-ground surfaces, slightly inclining to the enamel surface, of the cervical part of the mesiodense was prepared, etched with HCl and examined under the scanning electron microscope. The contents of seven elements (mass %) were analyzed quantitatively with an electron probe microanalyzer.
Results: The width of the rod sections at the cervical enamel was larger than that at the incisal edge enamel. The phosphorus, carbon and magnecium contents at the cervical and incisal edge enamels of the mesiodense were higher than those of the canine, while the calcium, oxygen and sodium contents of the mesiodense were lower than those of the canine.
Discussion: It is thought that the cervical enamel of the mesiodense is more easily decayed by dental caries than the incisal edge enamel. It is considered that the cervical enamel of the mesiodense is low calcified than that of the canine. It is thought that more calcium in other condition from hydroxyapatite exists in the cervical enamel of the mesiodense than that in the canine.
Conclusion: The difference was recognized in the histological structure and elemental composition of the cervical enamels among the mesiodense, canine, premolar and molar.

(COI: No)

P2-145

Abnormal enamel formation in thermosensitive TRPV channels knockout mice

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Dental enamel is the hardest tissue in the body and secreted by a specialized ameloblast. Although a great deal of progress in the knowledge of enamel formation has been made, the developmental step with gradual physical hardening remains unclear. Thermosensitive transient receptor potential vanilloid 3 and 4 (TRPV3, TRPV4) were known as non-selected Ca^{2+} permeable ion channels which are activated by warm temperatures. We hypothesized that TRPV3 and TRPV4 channels contribute to the development of ameloblast and calcification of dental enamel, and investigated the influence of TRPV3, TRPV4 on enamel formation in the wild (WT), TRPV3 and TRPV4 knockout (V3KO, V4KO) mouse. We immunohistochemically investigated tooth germs in postnatal day 5 and six-week-old-incisors and molars, using TRPV3 and TRPV4-specific antibodies. We found conspicuous TRPV3 and TRPV4-immunoreactivity in ameloblast layers in the WT tooth germs. There was no significant difference in tooth outlook, size and thickness of enamel between WT and V3KO, V4KO. Under the observation of SEM, we found immature development of the enamel prism and interrod substances in the incisors and molars from V3KO, V4KO mice compared with WT. These observations suggested that TRPV3, TRPV4 are localized in the ameloblast layer of tooth germs, and may affect enamel formation.

(COI: No)

P2-146

Histological and analytical studies on the role of melatonin in the structure and composition of teeth dentin

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The purpose of the present study is to examine the relationship between the structure and composition of teeth dentin and the role of melatonin through the histological and analytics studies. In this experiment, 5-, 6-, and 7-day old SD rats were used. These rats were divided into three groups: 1) a control group; 2) a low-concentration group; and 3) a high-concentration group. In the control group, two dark-staining incremental lines of hematoxylin and one light-staining layer were observed in incisor dentin. In the high-melatonin concentration group, this layer disappeared. The number and size of calcospherites in predentin increased in proportion to the concentration of melatonin administered. The new incremental line was confirmed in the incisor predentin and molar dentin of the melatonin treated groups. Ca and P content were increased in the melatonin treated group. It is considered that melatonin participates in the formation of incremental lines and the calcification mechanism of dentin.

(COI: No)

P2-147

Intercellular Odontoblast Networks via Extracellular Glutamate

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Various stimuli to odontoblasts induce sharp pain. Transient receptor potential (TRP) channels in odontoblast receive these stimuli, which induce the release of ATP to nearby odontoblasts and trigeminal ganglion (TG) neurons and establish intercellular signaling. Recently, it has been shown that odontoblasts express metabotropic glutamate receptor subtype 5. This implies that odontoblasts are capable of receiving extracellular glutamate. However, it remains unclear whether cells, such as neurons and/or odontoblasts themselves, release glutamate in dental pulp. We thus examined intercellular odontoblast-odontoblast and odontoblast-TG neuron signal transduction via glutamate. During mechanical stimulation of an odontoblast, not only the stimulated cell but also the nearby odontoblasts and TG neurons showed increases in intercellular free Ca^{2+} concentration ($[Ca^{2+}]_i$). After application of a cocktail of GluR antagonists, the responses in nearby odontoblasts and TG neurons were suppressed. When we applied each agonist for GluR subtypes to the odontoblasts, $[Ca^{2+}]_i$ increased. These results suggest that a mechanically stimulated odontoblast is capable of releasing glutamate, which activates synchronous intercellular Ca^{2+} signaling via GluR on the cluster of adjacent odontoblasts in an autocrine/paracrine manner. The released glutamate also activates TG neurons in dental pulp as a neurotransmitter. We propose that these inter-odontoblasts and odontoblast-TG neuron networks drive odontoblastic functions such as reactive dentin formation and/or augmentations in sensory signaling.

(COI: No)

P2-148

Ca^{2+} signaling activated by alkaline environment in rat odontoblasts

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Alkaline environment provided by calcium hydroxide, which is used for endodontic treatment, induces reparative dentinogenesis, however its precise mechanisms remain unclear. We examined intracellular Ca^{2+} signaling pathway induced by alkaline environment in rat odontoblasts. In dentin sialoprotein-positive acutely isolated odontoblasts, intracellular free calcium concentration ($[Ca^{2+}]_i$) was measured by fura-2 fluorescence. In the presence (2.5 mM) and absence (0 mM) of extracellular Ca^{2+}, application of alkaline solution prepared by adding NaOH increased $[Ca^{2+}]_i$, showing a dependence of $[Ca^{2+}]_i$ on extracellular pH (pH 8.5-10.5). In the presence of extracellular Ca^{2+}, $[Ca^{2+}]_i$ increase induced by the alkaline solution were greater than in the absence of extracellular Ca^{2+}. Alkaline solution-induced $[Ca^{2+}]_i$ increases were dependent on the extracellular Ca^{2+} concentration. Repeated applications of alkaline solution did not induce a desensitizing effect on the increase. In the presence of extracellular Ca^{2+}, $[Ca^{2+}]_i$ increases evoked by the alkaline solution (pH 10) were inhibited by HC030031, a specific antagonist of transient receptor potential ankyrin subfamily member 1 (TRPA1) channels. These results indicate that alkaline stimuli activate Ca^{2+} mobilizations via TRPA1 channels and intracellular Ca^{2+} release in odontoblasts, suggesting that alkali-sensing mechanisms in odontoblasts may play an important role in driving dentinogenesis induced by calcium hydroxide.

(COI: No)

P2-149

Voltage-dependent calcium influx pathway in rat odontoblasts

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Odontoblasts play a role in the sensory signal transduction involved in generating dentinal pain. We have previously reported that depolarizing-stimuli induce $[Ca^{2+}]_i$ increases; however, voltage-dependent Ca^{2+} current activity was not observed. Thus, the voltage-dependent Ca^{2+} influx pathway in odontoblasts is still unclear. Recently, calcium homeostasis modulator (CALHM1), which is able to carry Ca^{2+} and ATP voltage-dependently, has been found. In the present study, we hypothesized that CALHM1 contributes to the voltage-dependent Ca^{2+} influx and aimed to clarify its functional expression in odontoblasts. In acutely isolated odontoblasts, the application of high-K^+ extracellular solution elicited transient $[Ca^{2+}]_i$ increases in the presence of extracellular Ca^{2+}, but not in the absence of extracellular Ca^{2+}. The high-K^+-induced $[Ca^{2+}]_i$ increase showed dependence on extracellular Ca^{2+}. Application of Gd^{3+}, but not Ni^{2+}, inhibited the depolarization induced-increase in $[Ca^{2+}]_i$. The results indicated that membrane depolarization activates the Gd^{3+}-sensitive voltage-dependent transmembrane Ca^{2+} influx in odontoblasts, which was not mediated by Ni^{2+}-sensitive-T/R type voltage-dependent Ca^{2+} channels. The results imply that the voltage-dependent Ca^{2+} influx in odontoblasts might be mediated by Gd^{3+}-sensitive CALHM1, which may play an important role in the generation of receptor potential, elicited by the sensory transduction sequence during dentinal pain.

(COI: No)

P2-150

Involvement of The TRPV4 Channel in gingival epithelia barrier function

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Transient receptor potential vanilloid 4 (TRPV4), a nonselective cation channel, is reported to play a skin barrier function via direct interaction with beta-catenin, a component of cell-cell adhesion complex. We found TRPV4 expression in oral epithelia using RT-PCR analysis. Under immunohistochemical observation, conspicuous TRPV4 immunoreactions are found in junctional epithelium, which makes direct contact with tooth enamel. Junctional epithelium, surrounding tooth like an epithelial collar, supports epithelial attachment to the tooth, and at the same time, it is the location of periodontal inflammation. We found E-cadherin and beta-catenin with prominent actin rings in junctional epithelium in WT mice. However, the staining intensity was weak in TRPV4 knockout (TRPV4KO) mice. To explore whether TRPV4 affect periodontal disease, we investigated in the mice with ligature placement by sterile silk sutures. Under micro CT analyses, the decrease in bone volume in TRPV4KO mice with ligatures was greater than that of WT mice. Based on these results, TRPV4 is suggested to contribute to the epithelial barrier in tooth-epithelial junction and affects periodontal disease progression.

(COI: No)

P2-151

TRPV3 channel contributes to rapid wound healing in oral epithelia via EGFR signaling

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The oral cavity provides an entrance to the alimentary tract and serves as a protective barrier against a drastic variation of stimuli compared with other tissues. Oral mucosa is susceptible to injury, but it shows faster wound healing than the skin and less scar formation. However, the molecular pathways that regulate this wound healing are still unclear. Here we show that transient receptor potential vanilloid 3 (TRPV3), a thermosensitive Ca^{2+}-permeable channel activated by warm temperatures (>33℃), is functionally expressed in oral epithelia. We found delayed closure of wounds after tooth extraction in TRPV3-deficient (TRPV3KO) mice compared with that in wild-type (WT) mice. We also found that TRPV3 activation increased the number of proliferating cells and EGFR phosphorylation in primary cultured oral epithelial cells from WT, but they were not found in the cells from TRPV3KO. Additionally, the number of proliferating cells and phosphorylated EGFR expression in oral epithelia were also markedly reduced in TRPV3KO mice. These results suggest TRPV3 in oral epithelia promotes the proliferation of oral epithelial cells and contributes to rapid wound repair via EGFR phosphorylation.

(COI: No)

P2-152

Merkel cells transduce mechanical stimuli and release neurotransmitters

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Merkel cells (MCs) are thought to form a part of the MC-neurite complex with sensory neurons. However, the mechanism of neurotransmission between the MCs and nerve endings in this complex has not been clarified yet. We therefore prepared co-cultures of MCs and trigeminal ganglion (TG) neurons and recorded intracellular free Ca2+ concentrations ([Ca2+]i) in response to direct mechanical stimulation of the MCs. TG cells were isolated from 5-day-old Wistar rats and cultured in L-15 medium. MCs were acutely isolated from golden hamster (3-5 weeks old) buccal mucosa following intraperitoneal injections of quinacrine 24 h prior to isolation. These were added to the culture dish of TG cells. We identified quinacrine fluorescence-positive cells as MCs. Fura2 fluorescence was used to measure [Ca2+]i. Application of direct mechanical stimuli using a glass micropipette caused an increase in [Ca2+]i, which was dependent on the intensity of the mechanical stimuli and sensitive to antagonists of transient receptor potential (TRP) vaniloid subfamily member (V) 1, TRPV2, TRPV4, and TRP ankyrin subfamily member (A) 1 channels. In the co-culture system of TG cells and MCs, direct mechanical stimulation of the latter induced [Ca2+]i increases not only in the stimulated MC, but also in TG neurons. These results indicated that mechanical stimulation of MCs activates the TRPV1, TRPV2, TRPV4, and TRPA1 channels; sensory signals are then transmitted to the neurons through diffuse chemical substance(s).

(COI: No)

P2-153

Morphological and molecular characterization of microfold cells in nasopharynx-associated lymphoid tissue

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Mouse nasopharynx-associated lymphoid tissue (NALT) located at the base of the nasal cavity is a site for induction of mucosal immune responses against airway antigens. The follicle-associated epithelium (FAE) covering the luminal surface of NALT is characterized by the presence of microfold cells (M cells). M cell is a specialized epithelial cell that delivers luminal antigens to lymphocytes underneath epithelium. Although recent studies are uncovering the molecular aspects of M cell in intestinal Peyer's patch, little is known about NALT M cell. Here, we show that NALT M cells express glycoprotein 2 (GP2), M-Sec, Spi-B and Ccl9, which are fundamental molecules of Peyer's patch M cell. Receptor activator of nuclear factor kappa-B ligand (RANKL) is a strong inducer of M cells in the intestine. We found that RANKL is expressed by stroma cells underneath FAE of NALT, and administration of RANKL increased the number of NALT M cell, suggesting that RANKL regulates the differentiation of NALT M cell. Mouse NALT is considered as an equivalent of human Waldeyer's ring. Our research will contribute to understanding mucosal immune responses in nasopharynx.

(COI: No)

P2-154

Morphological approach for a paracellular fluid transport in salivary glands

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During muscarinic stimulation, the fluid secretion via the paracellular route dominants the whole fluid secretion. We applied multi-directional TEM observation for 3D reconstruction on freeze fracture replica, and quick 3D reconstruction of CLSM images. Using sulfo-rhodamine B or Lucifer Yellow in the perfusate, we observed the intercellular space via the fluorescence and the intercellular canaliculli as much less fluorescence. This system allowed us to obtain 30 sliced images every 2 s and produced 3D reconstructed images (5 Live, Zeiss).

Results: 1) Carbachol/isoproterenol stimulation opened the paracellular passage of Lucifer Yellow. 2) Simultaneously, the cytoskeleton lattice beneath the tight junction became narrower than that during control perfusion. 3) The 3D movie showed the increase in cell surface movement during carbachol stimulation. 4) During stimulation with carbachol, the cell volume decreased, shrinking the cytoskeleton fiber intervals, suggesting an increase in the cytoskeleton movement, and thus the strand particles (claudine) of the tight junction could increase the movement to allow paracellular movement of water and solutes. The present findings suggest that a part of the paracellular transport could be driven by plasma membrane vibration near the intercellular canaliculli. Acknowledgement: JSPS KAKENHI (23590271, 26460308).

(COI: No)

P2-155

Subcellular localization and functional implication of V-ATPase in ductal cells of mouse salivary glands

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V-ATPase, which is composed of V_1 (A-H) domain and V_o (a, c, c', c'') domain, is known to be localized in intracellular membranes of organelles of cells or in their cell membranes to acidify the outside. We previously reported that the B2 subunit isoform of V-ATPase was localized in the ductal cells of the mouse major salivary glands. In this study, we have further studied subcellular localization of V-ATPases and demonstrated that immunofluorecence for a1 subunit tend to be localized in the apical region, and that B2 subunit isoform was found in the apical and the basal regions of parotid ductal cells, and that immuno- transmission electron microscopy for V-ATPase was proved to be localized in the apical, lateral, and basal membranes and basal infoldings of striated duct. Phenotypes analysis of the knockout mice of the a3 subunit isoform (a3-KO mice) revealed that the size of salivary glands and the amount of saliva secretion in the a3-KO mice was reduced. The amount of saliva secreted by pilocarpine injection (i.p.) in the a3-KO mice was significantly less, whereas no difference was detected by isoproterenol. Additionally, intraoral salivary pHs in the a3-KO mice tended to be slightly acidified. These results suggest that the V-ATPase in salivary glands is localized in the apical membrane and the basal region of ductal cells and is involved in salivary pH adjustment and absorption by unknown mechanism.

(COI: No)

P2-156

Bone marrow-derived cells have the ability to differentiate into parenchymal cells of salivary glands

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In recent years, it has been reported that bone marrow-derived cells (BMDCs) were capable of differentiating into multiple cell types of many organs. Therefore the treatment by using BMDCs for the regeneration medicine is expected in the future. We examined whether BMDCs can differentiate into salivary gland cells in the mice. BMDCs from green fluorescence protein (GFP) mice were transplanted into irradiated syngeneic GFP-negative mice. One, two, three and six months after bone marrow transplantation, the salivary glands were removed and immunohistochemical examinations were carried out. Immunohistochemistry (IHC) for GFP showed that GFP positive cells were found in salivary glands. We performed the double-labeled fluorescence IHC staining to identify the cell type of GFP-positive cells, by using following antibodies: AQP as a marker for the acinar cells, cytokeratin 19 as a marker for the ductal cells and α-SMA as a marker for the myoepithelial cells. The double-labeled fluorescence IHC staining demonstrated that GFP-positive cells were detected as secretory cells, ductal cells and myoepithelial cells. These results suggest that BMDCs migrate to salivary glands and differentiate into parenchymal cells of salivary glands.

(COI: No)

P2-157

Putative Bio-Markers in saliva by microRNA pattern depending on secretion systems from salivary glands

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MicroRNAs (miRNAs) are small non-coding RNAs of 18-28 nucleotides that play key roles in the regulation of gene expression. To examine the possibility of miRNA in saliva as a Bio-Marker, expression patterns of miRNAs in submandibular glands (SMGs) and in whole saliva of ICR mice with various hormonal treatments were analyzed by quantitative real-time PCR.
SMGs were investigated for miRNAs and 42 miRNAs were identified. Among 42 miRNAs, miR-21a, miR-141 and miR-143 were much abundant in male mouse. Castration caused remarkable decrease in the expression of these three miRNAs. DHT administration to the castrated animals increased miR-21a, miR-141 and miR-143 such as male. Suggesting, these three miRNAs in the tissues were regulated by androgen.
In the case of exocrine whole saliva collected from mice stimulated parasympathetic nerve, miR-143 was not secreted to saliva, also miR-21a and miR-141 were secreted abundantly but were not dependent on androgen like as SMG tissues. These results suggest that amounts of miRNAs in saliva are not always correlated to ones in SMG tissues, thus depend on specific transport system such as exosome. Also, miR-15a, miR-16, miR-23a and miR-451a were secreted in female mice abundantly. Furthermore, miR-451a secretion was increased by ovariectomy extremely. It is known that miR-451a regulates the drug-transporter protein P-glycoprotein, potentially promoting resistance to the chemotherapy drug Paclitaxel (http://miRBASE.org/). We refer miR-451a in saliva is a putative bio-marker to indicate drug-transport rate for ovarian diseases.

(COI: No)

P2-158

Ultrastructural localization of endogenous peroxidase activity in secretory granule of rhinoceros parotid gland

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A parotid gland (PG) was obtained from a male rhinoceros aged 36 years. Specimens were fixed in 10% formalin for light microscopy. The same specimens were then subsequently fixed with 2% para-formaldehyde-2% glutaraldehyde and 1% OsO_4 buffered with 0.1M cacodylate buffer for electron microscopy. After dehydration, the tissue was embedded in Quetol 653. Using ordinary electron microscopy, acinar secretory granules of PG showed a bipartite structure consisting of the main portion and of the dense bodies (or cores). In the present study, nickel grids with ultrathin sections of Quetol 653 embedded blocks were incubated for 1h in a 3, 3'-diaminobenzidine tetrahydrochloride (DAB)-reaction solution consisting of 0.1% DAB and 0.01% H_2O_2 causing an endogenous peroxidase (PO) reaction. X-ray microanalysis of the DAB-reacted ultrathin sections was performed under an energy dispersive X-ray spectrometry (EDS) attached to a JEM 1400 Plus operated at 80 kv. EDS reflected the presence of moieties caused by the PO reaction. Therefore, the mapping patterns of nitrogen were restricted to the dense bodies. In the rat parotid gland, the PO reaction covered all portions of the acinar secretory granules including the typical serous secreting cells. These results suggest that the parotid gland of the male rhinoceros displayed the morphological characteristics of seromucous secreting cells.

(COI: No)

P2-159

Involvement of MARCKS phosphorylation in lipid rafts in amylase release in parotid acinar cells

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Myristoylated alanine-rich C kinase substrate (MARCKS) is known as a major cellular substrate for protein kinase C. The phosphorylated-MARCKS (p-MARCKS) translocates from the membrane to the cytosol. It has been thought that MARCKS has various cellular functions such as membrane trafficking. MARCKS has been implicated in the actin cytoskeleton regulation through the modulation of phosphoinositide in lipid rafts. In parotid acinar cells, the activation of β-adrenergic receptors provokes exocytotic amylase release. Here, we investigated the involvement of MARCKS phosphorylation in amylase release in rat parotid acinar cells. MARCKS protein in the acinar cells was detected. The β-agonist isoproterenol (IPR) induced MARCKS phosphorylation. IPR induced MARCKS translocation from the membrane to the cytosol. Lipid rafts, which were isolated as detergent-resistant membranes (DRMs), were separated by sucrose density-gradient centrifugation from the acinar cells lysed with 1% Triton X-100. MARCKS was found in monosialoganglioside GM1a-rich DRMs, but that in the DRMs markedly decreased by IPR stimulation. MARCKS-related peptide as the MARCKS inhibitor inhibited the IPR-induced amylase release. These results indicate that MARCKS phosphorylation is involved in amylase release in parotid acinar cells. MARCKS translocation from the lipid rafts to the cytosol may regulate exocytosis.

(COI: No)

P2-160

Morphological study of human submandibular duct: nerve distribution of sublingual caruncula, the common opening area

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Many studies report on the submandibular gland, but detailed structural studies of the human submandibular (Wharton) duct opening are rare. Out of the main salivary glands, the human submandibular gland secretes the most amount of saliva into a sublingual caruncula, an open area shared with the sublingual duct. This common opening area has not been the subject of many reports and its nerve distribution is still unclear. Sialolithiasis is known to be most common in the duct of the submandibular gland, usually causing pain from the sialolith in the duct. For certain, investigating the structure and nerve distribution of the submandibular duct has clinical importance as well. In this study, we conducted an immunohistochemical observation of the common opening area shared between the submandibular and sublingual ducts by using an antibody against anti protein gene product PGP 9.5, a specific marker for neurons, in order to study its nerve distribution. Seven materials were obtained from human adults ranging from age 74 to 93 from the Japanese cadaver collection at Kyorin University School of Medicine. After fixing the removed material in 4%PA/PBS overnight. They were washed with PBS, soak in 20% sucrose, and made into 15 μfrozen sections. Florescence microscope camera was used for observation. Results revealed an abundance of nerve fibers in the opening area of the Wharton duct, as well as an abundance of blood vessels surrounding the duct. We also confirmed the presence of smooth muscle inside the duct wall in the opening area.

(COI: No)

P2-161

Morphological Changes of Myoepithelial Cells in the Rat Submandibular Gland Following the Partial or Total Sialoadenectomy

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Objective: Myoepithelial cells (MECs) surround the basal surface of salivary gland acini. However, their function for salivary secretion is still unclear. Salivary secretion from the residual gland is considered to be accelerated when the glandular tissues were damaged or surgically excised. We analyzed morphological changes of MECs in residual and non-operated contralateral (NOC) submandibular glands after the partial or total sialoadenectomy.
Methods: Male Wistar rats of 8-weeks-old were used. Whole or distal-half of right submandibular gland was surgically excised. After 1, 2, 3 or 8 weeks of surgery, rats were fixed and residual and/or left NOC submandibular glands were prepared for frozen sections and immunohistochemistry using the polyclonal antibody against smooth muscle actin (SMA).
Results: In both residual and NOC glands, number of visible cell-bodies (nucleus and perinuclear region) of SMA-positive MECs and SMA-immunopositive area in serial sections increased significantly after the surgery. Three-dimensional analysis revealed that number, length and thickness of processes covering the acini were enhanced substantially. Complexity of process-branching measured by number of primary and terminal processes clearly was up-regulated in both residual and NOC glands.
Discussion: MECs adapt their morphology of their processes according to the demand of salivary secretion. MECs presumably promote salivary secretion by tightened their grasp on glandular acini.

(COI: No)

P2-162

The interaction between ACh and VIP on parasympathetic blood flow increase in rat sublingual gland

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Previously, we reported that the parasympathetic blood flow increase is evoked by both cholinergic and non-cholinergic fibers in sublingual gland (SLG). The parasympathetic vasodilation evoked by non-cholinergic fibers has been reported in orofacial area such as lip, tongue, masseter muscle, although the precise mechanisms of non-cholinergic parasympathetic vasodilation is still unclear. The SLG secretes mucous saliva including mucin, and it is well known that the vasoactive intestinal polypeptide (VIP) is important in protein secretion from acinar cells. Thus, in the present study, we examined the rule of VIP in parasympathetic blood flow increase of SLG. The urethane anesthetized rats paralysed by pancuronium bromide were artificially ventilated. The cervical vagi and cervical sympathetic trunk were cut in the neck bilaterally. The blood flow of SLG (SLGBF) were analyzed by laser speckle imaging flow meter when the central cut end of lingual nerve (LN) was electrically stimulated (20 V, 20 Hz, 20 s). The SLGBF increase evoked by LN stimulation was completely inhibited by intravenous administration of autonomic ganglion blocker hexamethonium. The SLGBF increase was reduced to 25% by simultaneous administration of atropine and VIP antagonist, although that was reduced to 60% by atropine only. The VIP or ACh administration induced the SLGBF increase, and the value of SLGBF increase was similar to that evoked by LN stimulation. Thus, it was suggested that ACh and VIP are related to parasympathetic SLGBF increase.

(COI: No)

P2-163

Morphological and histochemical features of the intercalated duct in the submandibular gland of mice deficient for the androgen receptor

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The submandibular gland (SMG) of mice has a marked sexual dimorphism, in which a special duct portion called granular convoluted tubule (GCT) develops from the striated duct (SD) preferentially in males. In the androgen-receptor-knockout (ARKO) male mice, the SMG appears similar to that in control and ARKO females. The administration of androgens to ARKO males had no effect on SMG, whereas the administration of thyroid hormone (T4) caused an extensive conversion of SD cells to GCT cells (Adthapanyawanich et al., in press). Another representation of sexual dimorphism in the SMG is the retaining of granular intercalated duct (GID) cells in the adult female mice. GID cells have secretory granules containing submandibular gland protein C (SMGC) and considered to be the remnants of terminal tubule cells that constitute the precursor of the acinar system of perinatal mice. In the present study, we found that the SMG of ARKO male and female mice also have many GID cells that are similar to those in control females. The administration of androgens to ARKO males had no effect on GID cells, but the administration of T4 caused marked decrease in the number of GID cells, as confirmed with immunohistochemistry for SMGC and electron microscopy. These results suggest that the development of GID cells is negatively regulated by AR, but that T4 can overcome the absence of AR by functioning downstream of the signaling pathway of androgens in the mouse SMG.

(COI: No)

P2-164

Dynamic change of PACAP receptor with the development of granular ducts in male mouse submandibular glands

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Saliva secretion is mainly controlled by autonomic nervous system. Pituitary adenylate cyclase activating polypeptide (PACAP) is now recognized as the multi-functional neuropeptide in various organs. We previously compared the distribution of PACAP receptor (PAC1R) in three major salivary glands of young and old male C57BL/6 mice. The distribution of PAC1R in the glands was not different by age. In submandibular gland, PAC1R was detected in the tall columnar epithelial cells, called pillar cells, in granular ducts and some of the cells in the striated ducts. The granular duct is characteristic in rodents. In this study, we examined the expression of PAC1R with the development of mouse submandibular gland. The submandibular glands at 1, 3, 5, 7 days-old and 2, 3, 4, 8 weeks-old male C57BL/6 mice were used for the immunohistochemical detection of PAC1R. Granular duct was not identified until 3 weeks after birth. PAC1R was detected in the striated duct by 2 weeks. At 3 weeks, granular duct was clearly identified and PAC1R was expressed in the pillar cells of the duct. After 4 weeks, PAC1R was more strongly detected at pillar cells than 3 weeks. These results indicated that the distribution of PAC1R was changed from striated duct to pillar cells with the formation of granular duct. A precise study might be necessary to clarify the function of pillar cells by examining the shift of PAC1R-positive cells in the submandibular gland.

(COI: No)

P2-165

Morphology and gene expression profile of the submandibular gland of androgen-receptor-deficient mice

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In the submandibular gland (SMG) of mice, the granular convoluted tubule (GCT) develops preferentially in males dependent on androgens. To clarify the molecular mechanism of androgen action in SMG, we examined the morphology and gene expression profile of the SMG of mice deficient for the androgen receptor (ARKO). The development of GCT and expression of GCT-specific products such as NGF were even lower in ARKO male SMG than in control female SMG. The administration of androgens to ARKO males had no effect on SMG, whereas the administration of thyroid hormone (T4) caused the extensive conversion of striated duct cells to GCT cells with the increase of NGF mRNA. Gene expression profiles in control and ARKO male SMG were analyzed by DNA microarrays, and genes with higher or lower expression in ARKO male SMG were determined. They were then classified into groups according to their responsiveness to the administration of dihydotestosterone (DHT) or T4 to ARKO males. RT-PCR revealed that, while no gene was responsive to DHT, expression of many genes was up- or down-regulated by T4. These results revealed that GCT cell differentiation induced by androgens is dependent on the classical androgen receptor (AR), whereas that by T4 is independent of AR, suggesting that T4 functions downstream of the action of androgens in the signaling pathway leading to GCT differentiation.

(COI: No)

P2-166

Intracortical interaction evoked by periodontal ligament nociception in rat

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The orofacial nociception is transmitted to the primary somatosensory cortex (S1) via the ventral posteromedial nucleus in thalamus. Several morphological studies have demonstrated that S1 is connected to the secondary somatosensory cortex (S2) and the area 5, but the functional organization among them is still unknown.
Recently, it was reported that electrical stimulation of the rat periodontal ligament elicited neural excitation in the region composed of ventral S2 and the insular oral region (IOR), which simultaneously occurred with S1 excitation. However, the physiological relationship between S1 and S2/IOR was unclear yet. To address this issue, we observed intracortical responses evoked by the electrical stimulation of rat periodontal ligament by *in vivo* optical imaging using voltage-sensitive dye.
Electrical stimuli in the periodontal ligament of a incisor evoked the simultaneous neural activation in S1 and S2/IOR. The amplitude and area of the responses increased over the stimulus intensity. The responses in S2/IOR were also observed by the electrical stimulation within the responsive area in S1 without no latency, and vice versa. After confirming those reciprocal interactions, we incised the cortical region between S1 and S2/IOR and then stimulated S2/IOR. Such operation resulted in disappearance of intracortical S1 activation.
These results suggest the existence of a dense intracortical connection between S1 and S2/IOR. This interaction has a possible role in processing the oral somatosensory information.

(COI: No)

P2-167

Catecholaminergic neurons involved in the neuronal system responsible for Cisplatin-induced nausea and/or emesis in the area postrema and the nucleus tractus solitarius

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To clarify if catecholaminergic neurons in the area postrema (AP) and the nucleus tractus solitarius (NTS) were involved in the induction of Cisplatin-induced nausea, we performed immunohistochemical analysis of c-Fos expression and catecholamine synthesis. Male Sprague-Dawley rats (200-300g) received intraperitoneal injection of Cisplatin (10.0 mg / kg body weight) or saline (5.0 ml / kg body weight). Animals were transcardially perfused with fixatives at 2, 6, 12, 24 and 48 hours after injection of Cisplatin or saline. Coronal sections (30 μm thick) were made from the brain removed from skull with a freezing microtome. In each section treated with a c-Fos antibody combined with either DBH (dopamine β-hydroxylase) or TH (tyrosine hydroxylase), immunoreactive neurons in the AP and the NTS were examined under a fluorescence microscope. Immunoreactivities for DBH and TH were found in a certain number of c-Fos immunoreactive cells in the AP and the NTS. These results suggest that catecholaminergic neurons in the AP and the NTS play an important role in drug-induced nausea. In animals fixed at 12 hours after injection of Cisplatin, the number of c-Fos positive AP neurons was significantly smaller than those in other rats fixed at different time course. This result indicates that the acute nausea and/or emesis may switch to the chronic symptom at around 12 hours after Cisplatin injection.

(COI: No)

P2-168

Jaw-position dependent surppression of the low threshold jaw-opening reflex during fictive mastication in rabbits

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The aim of present study is to verify whether the strong suppression of the low threshold jaw-opening reflex (Lo-JOR) at end of jaw-closing phase (end-CL) on the working side is fundamentally depends on jaw positions relative to configuration of jaw movement trajectories, or the physical distance from the intercuspal jaw position. We tested this question by using a removal appliance (splint) that increases inter-incisal distance during fictive mastication. EMG activity of the digastric muscle was recorded with movements of the incisor point of the mandible during fictive mastication. The jaw movement signal was used to deliver the stimuli to the inferior alveolar nerve at half of closing phase (hal-CL) and end-CL. The splint was unilaterally applied on the working side. The magnitude of the JOR suppression was 22.2% of control at end-CL and that was 62.2% at hal-CL under condition without the splint. When the inter-incisal distance was increased by application of the splint, the JOR was tested at a jaw position which was located at end of closing phase, but its physical dimensions were vertically and horizontally same as those of the hal-CL. It was found that magnitude of the JOR suppressions was significantly different between splint (41%) and hal-CL, suggesting that the strong suppression of the Lo-JOR at end-CL is fundamentally depends on jaw positions relative to configuration of jaw movement trajectories. In conclusion, this jaw position-dependent suppression of the JOR is advantageous for production of strong biting force on the working side.

(COI: No)

P2-169

Activation of α_2-adrenoceptors via cervical sympathetic nerve involves β-adrenergic vasodilation in the masseter muscle mediated by sympathoadrenal system

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Sympathetic activity is one of the important factors for the regulation of the hemodynamics of jaw muscles and disturbances in intramuscular blood flow evoked by modulation of sympathetic vasomotor response may be related to jaw muscle dysfunctions. Sympathoexcitation has been reported to cause changes of either increase or decrease of the blood flow in the masseter muscle (MBF). Although the reason for the differences in the effects is unclear, the interaction between neural and humoral mechanisms of MBF regulation may be important for the difference because sympathoexcitation induces activation of both sympathoadrenal system and cervical sympathetic nerves (cSN). We explored this question by investigating the effects of electrical stimulation of the splanchnic nerve (SPLN) on the MBF either intact or sectioning of cSN, and adrenoceptor agonists or antagonists on the responses in anesthetized rats. The SPLN stimulation caused a significant MBF increase and the increase significantly reduced by intravenous administration of propranolol. The MBF increase evoked by SPLN stimulation was almost abolished by cSN section. The SPLN stimulation after cSN section in combination with administration of clonidine significantly increased the MBF, but not phenylephrine. Our results indicate that cSN is involved in β-adrenergic vasodilation in the masseter muscle mediated by sympathoadrenal system, and suggest that the activation of α_2- rather than α_1-adrenoceptors via cSN contributes to this response.

(COI: No)

P2-170

Association of oral fat sensitivity with body mass index and food preference in Japan

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This study was conducted to evaluate the association between oral fat sensitivity and BMI among Japanese adults. We also aimed to evaluate the relation between oral fat sensitivity and taste preferences. The BMI and taste preferences of 25 healthy Japanese adults were investigated using measuring scales and a questionnaire. Sensitivities to prototypical tastants were determined as controls. Oral fat sensitivity was evaluated using oleic acid (OA) in non-fat milk. More than half of the participants detected OA in the non-fat milk at 2.8 mM and the OA detection threshold was associated with BMI. Based on the results, the participants were divided into three groups: super-hypersensitive (SHE), hypersensitive (HE), and hyposensitive (HO) group. No association was observed between the recognition threshold of each prototypical tastant and BMI. The average extent of preference for each prototypical tastant showed that only for sweet preference, significant differences were observed among the oral OA sensitivity groups. Moreover, we found a significantly higher preference for fatty sweet foods than non-fatty sweet foods in the SHE and HE groups but not in the HO group. These findings suggest that OA sensitivity is associated with BMI and the extent of preference for fatty sweet food in Japanese adults.

(COI: No)

P2-171

This poster presentation was withdrawn.

P2-172

Relationship between eating behavior and obesity in humans

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For a long time, obese people were thought to exhibit a different eating behavior (i.e., natural bite size, number of chews per bite, chewing speed, etc.) to normal weight people. However, actual relationship between obesity and eating behavior has not been fully elucidated. Therefore, we investigated the relationship between obesity and several individual eating behaviors (self-reported eating speed; ES, bite size, number of chews until final swallowing per bite; NCS, masticatory performance; MP) in 61 adult subjects (39 males; 22 females; mean age, 23.2 yrs). Fish sausage (FS) and bun (B) were used for the test food. Individual MP was measured by the gluco-sensor. Individual NCS for the FS or the B was counted by the masticatory counter. Body mass index (BMI) was calculated to estimate the individual degree of obesity. There was no significantly correlation between BMI and the MP. There was also no significant correlation between BMI and the NCS. On the other hand, significant positive correlation between the BMI and the bite size, and significant positive correlation between the BMI and the ES were obtained. These results suggest that the natural bite size and the ES closely relate to the obesity in humans.

(COI: No)

P2-173

Role of mitochondria in thymic epithelial cells

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Thymic epithelial cells (TECs) play an essential role in supporting T lymphocyte development and selection. TECs are functionally divided into cortical TECs (cTECs) and medullary TECs (mTECs) based on their localization within the thymic cortex or medulla, respectively. Mitochondria play a pivotal role in intracellular Ca^{2+} storage and ATP production. However, the relevance of mitochondria in the function of TECs has been unclear. To understand the role of mitochondria in TECs, we measured mitochondrial mass and their membrane potential in mouse TECs using MitoTracker Green and MitoProbe DiOC2(3), respectively. We found that the mitochondrial mass was larger in TECs than thymocytes in flow cytometry and confocal images. The mitochondrial mass was larger in mTECs than cTECs. Mitochondrial membrane potential was almost intact in cTECs, whereas a large fraction of mTECs had depolarized mitochondria. These data indicate that quantity and quality of mitochondria are different between mTECs and cTECs.

(COI: No)

P2-174

The Effect of Minodronate on Murine Hematopoiesis

Inoue, Satoshi; Arai, Hiroshi; Otsuka, Hirotada; Nakamura, Masanori (*Sch, Dent, Showa Univ, Tokyo, Japan*)

Bisphosphonates (BP) are potent inhibitors of osteoclast-mediated bone resorption, and are classified in the nitrogen-containing BP (NBP) and the non-nitrogen containing BP (non-NBP). We previously showed that one of NBP, alendronate (ALD), decreased the number of erythroid-lineage cells, increased the number of osteoclasts and granulocytes, and enhanced the cell size of osteoclasts *in vivo*, indicating that NBP might have a profound effect on murine hematopoiesis. Minodronate (MIN) is other NBP and is more potent inhibitor of bone resorption than alendronate. The purpose of this study is to clarify and compare the time-kinetic changes in hematopoietic cells and osteoclast by MIN and other non-NBP, clodronate (CLO). MIN (10 μmol/kg) or CLO (160 μmol/kg) were intraperitoneally injected into 8 weeks-old male BALB/C mice. In MIN group, whitish bone marrow and splenomegaly were observed 4days after the injection. Flow cytometric analysis of bone marrow indicated the decrease of the number of Gr-1-/CD11+ macrophage at 1-2days, and the increase of the number of Gr-1+/CD11+ granulocytes, the decrease of the number of TER-119+ erythroid cells and the recovery of the number of Gr-1-/CD11+ macrophages to the control level at 4days after the treatment. Histological study indicated the decrease of the number of TRAP+osteoclasts. These cells were located along the trabecular bones beneath the growth plate. These results indicate that hematopoietic cells are strongly influenced by MIN similar to ALD and suggest the different effect on osteoclasts from ALD.

(COI: No)

P2-175

Genenration and differentiation of iPS cells derived from plasminogen activator inhibitor-1 deficient patient

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Plasminogen Activator inhibitor-1 (PAI-1) is the key regulator of plasminogen activation system. Number of studies have shown the relationship between PAI-1 expression levels and diseases such as thrombosis and poor prognosis of cancers. Recently we have reported a PAI-1 deficient patient having apparent phenotypes of severe bleeding and impaired wound healing, both of which are not seen in the PAI-1 deficient mice. To investigate the intrinsic function of PAI-1, iPS cells from the patient were generated, and are differentiated into endothelial cells (ECs) which PAI-1 is mainly producing and having analyzed its role. After co-cultured with stromal OP9 cells, we isolated ECs by MACS with VEGFR2 antibody. The expression of some of the ECs markers were increased, which was more prominent in PAI-1 deficient iPS cells than in wild type. Furthermore we found that ECs from PAI-1 deficient iPS cells detached from dish bottom earlier than control, when the cells were cultured for longer period of time. These results suggest that PAI-1 plays critical roles in differentiation and maturation of ECs. We are now trying to confirm further authentic roles of PAI-1 in ECs and other kind of cell differentiation and functions, such as adipose cells and platelet/megakaryocytes.
(COI: No)

P2-176

EphA2 receptor and ephrin-A1 ligand expression in the spleen

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The spleen filters the blood, and red pulp macrophages are engaged in the phagocytosis of damaged erythrocytes. Recently it has been revealed that monocytes reside in the red pulp of the spleen more than in circulation and emigrate to inflammatory sites (Swirski et al., Science, 2009). We have studied whether EphA2 receptor and ephrin-A1 ligand in vascular endothelial cells could involve in the transendothelial migration of monocytes/macrophages, certain types of which clearly express these membrane proteins. In the present study we therefore examined EphA2 receptor and ephrin-A1 expression and localization in the mouse spleen to determine whether the organ has niches suitable for studying EphA2/ephrin-A1 functions in the transendothelial migration and colonization of monocytes/macrophages. RT-PCR analysis showed that substantial amounts of EphA2 and ephrin-A1 mRNA were expressed in the spleen of Balb/c adult male mice and significantly upregulated in the mice intraperitoneally treated with clodronate liposomes (FormuMax Scientific), which induce the depletion of phagocytes in vivo. Immunofluorescence analysis showed that (1) EphA2 expression was restricted in the red pulp: clearly in CD144-positive cells (splenic sinus endothelial cells) and ER-TR7-positive cells (red pulp fibroblasts) and (2) ephrin-A1 was expressed clearly in endothelial cells of the red pulp and marginal zone. These findings may indicate that the spleen is a suitable organ to examine EphA2/ephrin-A1 functions in terms of the transendothelial migration of monocytes and the colonization of macrophages.
(COI: No)

P2-177

Regulation of the blood-cerebrospinal fluid barrier permeability by TRPV4

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The blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB) restrict the diffusion of materials from systemic circulation to the central nervous system (CNS). Choroid plexus epithelial cells (CPECs) of the brain ventricles constitute the BCSFB, and regulate the infiltration of plasma proteins as well as immune cells into the interstitium of the CNS. While some pathological conditions are known to alter the barrier function of BCSFB, the regulatory mechanism is not fully understood. Here, we investigated the function of TRPV4, a polymodally gated divalent cation channel in CPECs. TRPV4 was localized broadly on the apical membrane in swine CPECs. Treatment with the TRPV4-specific agonist, GSK1016790A, induced a robust calcium influx and an immediate serine/threonine protein phosphorylation. In 10-20 min after the agonist treatment, a marked decrease in the amount of filamentous actin, and disintegration of the cell junctions were induced, while the protein levels of some tight junction proteins, ZO1 and Claudin 1, were unchanged. By contrast, inhibition of the basal TRPV4 activity with the TRPV4-specific antagonist, HC067047, reduced the basolateral-to-apical transport of alpha 2 macroglobulin. Overall, this study demonstrated a novel physiological function of TRPV4 in the regulation of BCSFB permeability.
(COI: No)

P2-178

Broad distribution of LYVE-1-expressing endothelial cells and reticular cells with special reference to the reticulo-endothelial system (RES)

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LYVE-1, a receptor molecule for hyaluronic acid, is selectively expressed in the lymphatic endothelium and some macrophage lineages. Besides the lymphatic endothelium, hepatic sinusoidal endothelial cells are known to express LYVE-1 and may function in the uptake of hyaluronate circulating in the body. Our immunohistochemical study revealed more broad distribution of LYVE-1 in the endothelium of the lung, adrenal gland, spleen, and heart (endocardium of auricle) of mice. In addition, reticular cells in the medulla of the lymph node intensely expressed LYVE-1. These cells are largely classified as reticulo-endothelial system (RES) for eliminating foreign particles. The LYVE-1-immunoreactive cells were topographically associated with a dense distribution of macrophages in each tissue: Kuppfer cells in the hepatic sinusoids, alveolar macrophages in the lung, macrophages within both sinusoidal lumen and parenchyma of the adrenal gland, macrophages in the splenic red pulp, and macrophages in auricular wall. Ultrastructurally, the immunogold particles for LYVE-1 were localized on the plasma membrane of all cell types. Function of the LYVE-1-expressing cells may be uptake of hyaluronate circulating in blood and lymph and subsequent degradation in relay with adjacent macrophages. This idea is partially supported by a higher activity of hyaluronidase in some organs possessing LYVE-1-expressing endothelial and reticular cells: the liver, lung, adrenal cortex, and medulla of the lymph node abundantly expressed mRNA of hyaluronidases.
(COI: No)

P2-179

Podoplanin$^+$ cells have roles of wound healing by expressing CCL2 and MMP9

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Recently, we have reported that a large number of stromal cells appeared and showed positive for podoplanin (PDPN), known as a lymphatic marker, in wound areas. In this study, we characterized the PDPN$^+$ cells appearing during wound healing in the mouse tongue to clarify their roles in tissue repair. We made a 1mm-deep laceration with a sharp sterile razor on the tongue in C57BL/6 mice under anesthesia. Tongues were excised and then snap-frozen at various times after injury. Their cryosections of 10-14 μm thickness were made for various morphological analyses. The epitherium completely healed by day5 after injury. The granulation was formed in the submucus where many active fibroblast-like cells were populated and formed fine meshworks without any tubular formation of lymphatic vessels. In addition, these fibroblast-like cells strongly expressed PDPN, but not LYVE-1. To examine the role of PDPN$^+$ cells, sections were multiple immunostained PDPN in combination with CD68, collagen type IV, CCL2 and MMP9. Most of PDPN$^+$ cells co-expressed CCL2 during wound healing and many CD68$^+$ cells migrated around the PDPN$^+$ cells. And PDPN$^+$ cells were stretched their processes towards collagen type IV$^+$ epithelial basement membrane. In addition, these PDPN$^+$ cells expressed MMP9 and the cell projections seemed penetrate through the basement membrane. These results suggest that the PDPN$^+$ cells in the wounded tongue may have some roles in wound healing by recruiting other cells such as CD68$^+$ cells with CCL2 and by expressing MMP9 to remodeling the tissue.
(COI: No)

P2-180

Preserved polycythemia in mice under long-term hypoxia

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Hypoxia is known to induce polycythemia caused by the activation of erythropoiesis mediated by increased erythropoietin (EPO) production. However, the elevation of EPO is limited and levels return to normal ranges under normoxia within one week of exposure to hypoxia, whereas polycythemia continues for as long as hypoxia persists. We investigated erythropoiesis in bone marrow and spleens from mouse models of long-term normobaric hypoxia (10% O_2) to clarify the mechanism of prolonged polycythemia in chronic hypoxia. The numbers of mature erythroid progenitors (CFU-E) in the spleen remarkably increased along with elevated serum EPO levels indicating the activation of erythropoiesis during the first week of hypoxia. After two weeks of hypoxia, the numbers of CFU-E returned to normoxic levels whereas polycythemia persisted for > 140 days. Flow cytometry analysis revealed a prolonged increase in the numbers of TER119-positive cells (erythroid cells derived from pro-erythroblasts through mature erythrocyte stages), especially the TER119 (high) CD71 (high) population, in bone marrow. The numbers of Annexin-V-positive cells among the TER119-positive cells particularly declined under long-term hypoxia, suggesting that the numbers of apoptotic cells decrease during erythroid cell maturation. These findings suggested that decreased apoptosis of erythroid cells during erythropoiesis contributes to presereve polycythemia in mice during chronic exposure to long-term hypoxia.
(COI: No)

P2-181

Ephrin-A1 Signaling in Monocytes/Macrophages Regulates Transendothelial Migration

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Eph receptors and ephrin ligands were membrane proteins that are implicated in cell adhesion and migration. We have investigated EphA2 and ephrin-A1 expressions and functions in monocytes (MOs), macrophages (Mϕs) and endothelial cells (ECs), and found that these mRNAs were expressed in ECs and MOs/Mϕs, and TNF α stimulated EphA2 and ephrin-A1 expressions in ECs. In the present study we examined whether ephrin-A1 signaling in MOs/Mϕs engages in the transendothelial migration. We used a MO/Mϕ cell line J774.1 and human vascular ECs. We established J774.1 cell lines with stable ephrin-A1 gene knock down by the shRNAs (efn-A1-KD J774.1). We found that Protein G-beads coupled with EphA2-Fc or ephrin-A1-Fc (EphA2-Fc-beads, ephrin-A1-Fc-beads) adhered to the ECs surface and ephrin-A1-Fc-beads were mostly invaginated to the cytoplasm of ECs, where actin filaments covered the beads. Moreover, ephrin-A1-Fc-beads induced membrane retraction in ECs. We also found that J774.1 parent cells adhered EphA2-Fc or ephrin-A1-Fc protein-adsorbed surface significantly higher in cell density than Fc protein-adsorbed surface. J774.1 cells on the EphA2-Fc and ephrin-A1-Fc surface formed cytoplasmic microspikes more prominently than those on the control surface although microspikes were less prominent in efn-A1-KD J774.1 cells on the EphA2-Fc adsorbed surface. Efn-A1-KD J774.1 cells seeding on confluent ECs migrated through the EC layer less frequently than J774.1 parent cells. These results may indicate that ephrin-A1 signaling in MOs/Mϕs and EphA2 signaling ECs are deeply implicated in transendothelial migration.

(COI: No)

P2-182

Lymphocyte homing ligand expression profile in the murine celiac and gastric lymph nodes

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Lymphocyte homing is mediated by interactions of L-selectin with their ligands and of integrin α4β7 with mucosal addressin cell adhesion molecule-1 (MAdCAM-1). L-selectin ligands are predominantly expressed on HEV in the cutaneous draining peripheral lymph node (PLN), whereas MAdCAM-1 is primarily expressed on HEV and lamina propria venules in mucosa-associated lymphoid tissue (MALT) such as Peyer's patches (PP) and is also expressed in the gut draining mesenteric lymph node (MLN). Celiac and gastric LNs and other secondary lymphoid tissues of ICR mice were stained immunohistochemically using anti-peripheral lymph node addressin mAb (MECA-79), anti-MAdCAM-1 mAb (MECA-367), and anti-GlyCAM-1 Ab (CAMO2). Celiac and gastric LNs HEVs were stained with MECA-79 and CAMO2, while MECA-367 staining is weak and/or absence on the majority of HEVs, especially in celiac LN. Although celiac LN, which is draining stomach and liver, therefore, belongs to MALT, the homing ligand expression profile is different from that in MLN.

(COI: No)

P2-183

Differential Expression of Toll-like Receptor-2, -4 and -9 in the Various Type of Epithelia Associated with Rat Peyer's Patches

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This study aims to elucidate the characteristic expression of TLR-2, -4 and -9 in follicle-associated epithelium (FAE) and epithelium of follicle-associated intestinal villi (FAIV) in rat Peyer's patches.

MATERIALS AND METHODS: The ileal cryosections with Peyer's patches from 5 male Wistar rats were stained with enzyme immunohistochemical method using anti TLR-2, -4 or -9 antibody.

RESULTS: TLR-2 was immunopositive in the striated borders of epithelia of ordinary intestinal villi (IV), anti-follicular side of FAIV (oFAIV) and follicular side of FAIV (iFAIV), but TLR-2$^+$ columnar epithelial cells in iFAIV were less frequent than those in IV and oFAIV. The immunopositive intensity of TLR-2 in the iFAIV was weaker than those in the IV and oFAIV. TLR-2, -4 or -9 was immunopositive in the apical membranes of many M cells in FAE. TLR-2$^-$ epithelial cells in the apical portions of lymphatic follicles were more numerous than those in the apical portions of IV and FAIV. No TLR$^+$ columnar epithelial cells were observed in the vicinity of TLR-2$^+$, -4$^+$ or -9$^+$ M cells.

DISCUSSION: From the present findings, the differential abilities of recognization to bacterial ligands among 3 types of epithelia and the cellular differentiation into M cells in FAE are discussed.

(COI: No)

P2-184

Anal tonsillar histopathology after the HRP and LPS administration. in the laboratory shrew, Suncus murinus

Sakai, Kazuyoshi[1]; Nomura, Ryuji[2]; Shinzato, Masanori[3]; Nishii, Kazuhiro[1]; Katoh, Yoshimitsu[1]; Hasegawa, Yoshimi[2]; Yamada, Kouji[4] ([1]*Sch. Health Sci. Fujita Health Univ., Toyoake, Aichi, Japan;* [2]*Anatomy, School of Medicine, Fujita Health University;* [3]*Pathology, School of Health Sciences, Fujita Health University;* [4]*Phsiology, School of Health Sciences, Fujita Health University*)

It has been elucidated that the laboratory shrew belongs to one of the ancestors of mammals. We reported that the laboratory shrew had tonsil-like structures near urogenitoanale, which were situated at different sites from any mucosal structures, named anal tonsil of mucosal immune structures. In order to research the function of anal tonsils, we administrated horseradish peroxidase (HRP) and Lipopolysaccharide (LPS) to the anal tonsils, and observed the change in the movement of inflammatory cells. After five, ten, fifteen and twenty minutes later of HRP administration, we took out the anal tonsil and made cryostat sections. These sections were reacted with peroxidase, and counted the number of the positive cells. After five minutes, there were several positive cells in the epithelium. We instilled 5mg/kg LPS into anal tonsils, and three days later, did same dosage again. After ten days later, we sacrificed animals, made paraffin sections. These sections were stained by CD3, CD19, CD20, CD1a S-100, FDC, lysozyme antibodies using immunostaining method. Especially, paracortico area of the tonsils, T-lymphocytes, dendritic cells and macrophages were increased.

(COI: No)

P2-185

Role of FABP7 in fat diet induced non-alcoholic fatty liver disease (NAFLD)

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Background: It has become evident that adipose tissue macrophages and liver macrophages (Kupffer cells, KCs) are important not only in local inflammation and tissue damage but also in systemic diseases associated with metabolic abnormalities. Recently, we reported that fatty acid binding protein (FABP) 7, a member of the intracellular lipid binding protein family, is expressed by KCs and regulates cytokine production and phagocytosis against dead cells. In this study, we investigated the role of KC-expressed FABP7 in the high fat diet (HFD) induced non-alcoholic fatty liver disease (NAFLD).

Methods: C57BL/6 (WT) and Fabp7-knock out (KO) mice were fed HFD (60 % kcal from fat) from 7 weeks until 19 weeks. Oil-red O staining was performed to detect lipid accumulation in the liver. To examine the liver injury and/or liver inflammation induced by HFD feeding, plasma ALT levels and mRNA expression of inflammatory cytokine/chemokine (TNF-α and MCP1) in liver were measured. For morphological analysis of KCs, F4/80 immuno-staining was performed.

Results: In KO mice, lipid accumulation in liver after HFD feeding was significantly decreased compared to WT mice. Plasma ALT levels and mRNA expression of TNF-α and MCP-1 in liver were lower in KO mice after HFD feeding than WT mice. The appearance of crown like structures, the aggregated KCs surrounding dead hepatocytes, was decreased in KO liver compared to WT liver.

Conclusion: Taken together, FABP7 expressed in KCs might be involved in the progression of NAFLD by modulating hepatic lipid metabolism.

(COI: No)

P2-186

The study of fat associated lymphoid clusters and hemal node-like structures in critical anemic mice

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The association between adipose tissue and immune system has been discussed, and fat associated lymphoid clusters (FALCs) are considered as a source of immune cells such as NK cells and macrophages. We previously reported induction of hemal node-like structure in omentum of critical anemic mice. Here, we analyzed the relationship between FALCs and hemal node like structure.

Splenectomized mice were treated with nitrogen-containing bisphosphonates to inhibit erythropoiesis in bone marrow, followed by injection with phenylhydrazine to induce hemolytic anemia.

In histological and immunohistochemical examination, numerous lymphoid cells formed clusters in the omental adipose tissue, and the most cells were B220-positive B lymphocytes. The CD3-positive T lymphocytes and F4/80-positive macrophages were dispersedly observed in these clusters. Furthermore, in critical anemic condition, TER119- and/or CD71- positive erythroblasts accumulated in these clusters. In RT-PCR analyses, we detected the expression of some mRNA related in hematopoiesis in hemal node-like structures, and Scf, Mcsf and SDF-1 were also detected in the omentum including FALCs.

These results indicated that the FALCs such as omental milky spots has the potency of supporting the microenvironment for hematopoiesis because of constantly providing the site of hematopoietic cells establishment. Therefore, we consider the possibility that some hematopoietic precursor cells establish and differentiate to erythroid progenitors in the FALCs, which may develop to hemal node-like structures under the emergency condition such as high EPO.

(COI: No)

P2-187

IL-17A inhibits osteoclast differentiation of RANKL-stimulated RAW 264.7 cells

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Periodontitis is a chronic inflammatory disease characterized by alveolar bone resorption. Inflammation-mediated bone loss is a major cause of various bone diseases, such as chronic periodontitis, and is due to an imbalance in bone remodeling that favors resorption. This imbalance is caused by increased inflammatory cytokines. Interleukin-17A (IL-17A) is a proinflammatory cytokine that is mainly secreted by activated T cells. IL-17A stimulates osteoclastic bone resorption via osteoblasts by inducing the expression of the receptor activator of NF-κB ligand (RANKL). However, little is known about the direct effects of IL-17A on the osteoclast precursors. We confirmed that IL-17A suppresses the osteoclast differentiation of RAW264.7 cells in the presence of RANKL in a dose-dependent manner. We also found that treatment with SP600125, a specific inhibitor of c-Jun N-terminal kinase (JNK), significantly inhibits the TRAP activity of RAW264.7 cells, which were stimulated by RANKL. In addition, we found that IL-17A reduces the phosphorylation of JNK and expressions of c-Fos, which were increased by RANKL stimulation. These results suggest that IL-17A-induces inhibition of JNK phosphorylation and that expression of c-Fos may be one of the factors that suppresses the differentiation of osteoclast precursors into osteoclasts.

(COI: No)

P2-188

Electron microscopic observation of a novel cytoplasmic rods and rings structure recognized by autoantibodies from patients with chronic hepatitis C viral infection

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Background and Purpose: Ribavirin binds to cellular inosine monophosphate dehydrogenase (IMPDH) and inhibits DNA synthesis. A combination of ribavirin and interferon-alpha is a standard therapy for chronic hepatitis C virus (HCV) infection. Autoantibodies that bind to a novel cytoplasmic rods and rings structure (RR) are induced in ~20% of the patients receiving this therapy. Ribavirin induces RR in nearly 100% of culture cells within 3h. The morphological feature of the RR was pursued by transmission electron microscopy (EM).
Methods: RR was induced in HeLa cells within 3 hour by ribavirin. Cells were stained with anti-RR/IMPDH (+) HCV sera or rabbit anti-IMPDH2 antibodies and developed using DAB. Slides were then fixed with glutaraldehyde and osmium double fixation. Samples for EM were prepared by epon embedding with a handstand gelatin capsule method.
Results: By immunofluorescence, many rods (3~10 μm length) and rings (2~5 μm diameter) were observed in ribavirin-treated cells, mostly in cytoplasm but some were also seen in nuclei. By EM, RR was morphologically a single paracrystalline array of individual filaments similar to intermediate filaments, without limiting membrane. Some rod structures were observed in the undetermined homogeneous structure in cytoplasm.
Conclusion: The fine RR structure morphologically appears to be a single paracrystalline array of individual filaments similar to intermediary filaments by EM.

(COI: No)

P2-189

Fabrication of tissue-engineered human arterial constructs by cyclic hydrostatic pressure

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Background: Ischemic heart disease is the primary cause of death and small diameter biological artificial vessels has been desired for supplying blood into ischemic lesion. Since hydrostatic pressure has been shown to increase extracellular matrices which are critical for arterial integrity, we aimed to fabricate biological arterial constructs using the apparatus generating cyclic hydrostatic pressure.
Methods and Results: Human umbilical arterial smooth muscle cells (HUSMCs) suspended in 10% fetal bovine serum/DMEM at the density 6.5×10^5 cells/mL, followed by culture in 5 mL syringe. Cyclic hydrostatic pressure was applied to HUSMCs in 5 mL syringe for 18 h. When HUSMCs were exposed to 110kPa-180kPa hydrostatic pressure at 0.002Hz, HUSMCs were self-assembled and exhibited sheet-like construct (2 mm × 4 mm × 100 μm), whereas pneumatic pressure control did not produce HUSMC sheet. The other cycle conditions, i.e., 0.25, 0.05, 0.01Hz, produced smaller- and irregular-shaped HUSMC sheets. In the HUSMC sheets fabricated by 0.002Hz hydrostatic pressure, mRNA expression of elastic fiber-related genes including fibrilin1, fibrilin2, fibronectin, fibulin4, and lysyl oxidase was more than two-times higher than in pneumatic pressure control ($p<0.05$, n=4-8).
Conclusions: hyper-hydrostatic pressure with lower cycle stimulation produced self-assembled human arterial sheets.

(COI: No)

P2-190

Effect of Epac-specific inhibitor, ESI-09 on heart failure model mice

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Although β-blockers are the first-line drugs for heart failure treatment, its overdose often leads to exacerbation. Recently, a downstream effector of β receptor, exchange protein (Epac) directly activated by cAMP emerges as a novel therapeutic target. Our previous studies showed that genetic disruption of Epac1 protects heart from pressure-overload as well as chronic catecholamine stress. Recently, Epac-specific inhibitor ESI-09 has been developed. In this study, we examined the effect of ESI-09 against heart failure. We performed chronic isoproterenol (ISO) infusion via osmotic mini-pump (60 mg/kg/day for 7 days) in C57BL/6 mice to produce heart failure model, and treated them with ESI-09 (1 or 5 mg/kg/day for 7 days) via intraperitoneal injection in one mice group. We found that left ventricular ejection fraction (LVEF) was significantly decreased in ISO infusion (from 65.3 ± 2.9 to 46.5 ± 2.9 %, $p< 0.01$), but ESI-09 did not improve the ISO-induced decrease of LVEF (from 66.4 ± 0.8 to 50.9 ± 2.3% at 1 mg, from 64.4 ± 2.6 to 51.3 ± 5.4% at 5 mg ESI-09). Moreover, injection of ESI-09 was also unable to inhibit ISO-induced cardiac hypertrophy and fibrosis. Rather negative results contrary to knockout study suggest the limitation for usage of this drug. ESI-09 has a non-selective effect to be capable of inhibiting both Epac1 and Epac2. Although we have to await for development of new specific drugs, our present results suggest that ISO-induced progression of heart failure includes a complex pathway, which cannot be simply prevented only by drug inhibition of Epac.

(COI: No)

P2-191

Intermittent hypoxia protects against oxidative stress-induced cell death by alteration of intracellular zinc regulation in adult rat cardiomyocyte

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Intermittent hypoxia (IH) with repetitive hypoxia-normoxia cycles has been shown to exert preconditioning-like cardioprotective effects. There are many findings demonstrated that IH against I/R injury via preserve ion homeostasis, including K^+, Na^+ and Ca^{2+}. Zn^{2+} is an important trace element in cellular physiology which including proliferation, cell signaling, metabolism and survival. However, there are very few literatures reporting the relationship between Zn^{2+} homeostasis and cardioprotection in IH process. The aim of the present study is to determine whether IH process changes intracellular Zn^{2+} homeostasis and which is involved in IH-induced cardioprotection. We investigated the changes in Zn^{2+} homeostasis using the Zn^{2+}-specific fluorescent dye, FluoZin-3. Using 2, 2'-dithiodipyridine (DTDP), a reactive disulphide compound that induce the intracellular release of Zn^{2+} and trigger cell death. In this this study, we found that DTDP release Zn^{2+} from MT, therefore elevated intracellular Zn^{2+} entry mitochondria via Ca^{2+} uniporter. Subsequently, mitochondrial Zn^{2+} increased to induce mitochondrial membrane potential depolarizeation and cell death. However IH increased mild ROS generation time-dependently to release Zn^{2+} from MT gently. This phenomenon lead to IH against DTDP induced cell death. These finding suggest IH induced Zn^{2+} store decrease, which attenuated excess intracellular Zn^{2+} induced cell death in cardiomyocyte.

(COI: No)

P2-192

Mathematical model of Ca^{2+} induced Ca^{2+} release in ventricular myocyte

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A human ventricular cell model including excitation-contraction coupling (HuVEC model) was developed in which the mechanisms of Ca^{2+} induced Ca^{2+} release (CICR) were largely refined. The CICR model is based on Hinch model (2004). In HuVEC model, the steep [Ca^{2+}] gradient near the Ca^{2+}-releasing sites was successfully generated as suggested experimentally. The voltage clamp simulation demonstrated that this local Ca^{2+} accumulation caused interaction among Ca^{2+} releasing units (CaRUs) and realized the graded Ca^{2+} release proportional to LCC. In the normal excitation-contraction coupling, the activation rate of a couplon was low at the onset of AP, and the following rapid rising phase of activation occurred after an apparent delay of a few milliseconds. During this delay, the activation was progressively accelerated through the Ca^{2+} accumulation in junction space in HuVEC model, which corresponded to the spread of individual RyR activations within a cluster of RyRs in full stochastic models of CICR reported recently. The inherently regenerative CICR was terminated through the decline in the activation rate, which was caused by the local Ca^{2+} depletion in SR.

(COI: No)

P2-193

Phase-2 reentry induced from decease in the cardiac sodium channel expression: in silico study

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Ventricular fibrillation in Brugada syndrome is thought to be associated with loss of function mutation in cardiac sodium channels. However, the relation between the fibrillation induction and the loss of function in sodium channels remains unclear. We have recently reported that decrease in sodium channel expression from the lateral surface membrane of each ventricular myocyte in a myofiber model incorporating the electric field mechanism, taking into account the intercellular cleft potentials, leads to the loss of function in the sodium current. Here, we extended the simulation to the initiation of phase-2 reentry in Brugada syndrome. We performed computer simulations of excitation conduction in the myofiber model, and investigated the effects of spatial and subcellular sodium channel distributions on the phase-2 reentry induction. In the myofiber model, the spatial heterogeneity of sodium channel did not reproduce the phase-2 reentry. In the same myofiber model but with specific subcellular sodium channel distribution, markedly decreasing in sodium channel expression of the lateral cell membrane of each myocyte, the spatial heterogeneity of sodium channel resulted in early repolarization followed by phase-2 reentry. Subcellular sodium channel distribution together with the spatial heterogeneity of sodium channel might be responsible for fibrillation induction in Brugada syndrome.

(COI: No)

P2-194

Dynamical Mechanisms of Early Afterdepolarizations in Long QT Syndromes: Insights from slow-fast decomposition analyses for human ventricular myocyte models

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Early afterdepolarizations (EADs) are known to cause lethal ventricular arrhythmias in long QT syndromes (LQTS). The aim of this study was to elucidate the mechanisms of EAD generation in LQTS by slow-fast decomposition analyses based on bifurcation theory. We have developed LQTS type 1 (LQT1) and 2 (LQT2) model cells from the mathematical models of human ventricular myocytes (Kurata et al, Biophys J, 2005; O'Hara et al, PLOS Comput Biol, 2011), assuming the inhibition of the delayed-rectifier K^+ channel current (slow component I_{Ks} or rapid component I_{Kr}). Roles of ionic currents in EAD generation were theoretically investigated by constructing bifurcation diagrams for fast subsystems as functions of slow variables. Slow activation gate of I_{Ks} or slow inactivation gate of the L-type Ca^{2+} channel current I_{CaL} were identified as slow variables (slow subsystems) during EAD generation. Bifurcation diagrams as functions of slow variables for fast subsystems showed stable equilibrium points (EPs) at depolarized potentials and EP destabilization via Hopf bifurcations with increasing the slow variables. Limit cycles, emerging via Hopf bifurcations, disappeared via homoclinic bifurcations as the slow variables further increased. EADs can be regarded as transient oscillations of the full system trajectories around the stable and unstable EPs in the vicinity of the bifurcation points during slow changes of the slow variables.

(COI: No)

P2-195

Electrophysiological characterization of a novel *SCN5A* mutant identified in a Brugada syndrome patient

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Cardiac Na^+ channel encoded by *SCN5A* ($Na_V1.5$) gene contributes to the depolarization phase of an action potential and is essential for maintaining cardiac rhythmicity. Some previously reported mutations of *SCN5A* are reported to cause Brugada syndrome. We identified in a Brugada syndrome patient a novel mutation of *SCN5A* which results in a K817E substitution at the voltage sensor region in the domain II of the channel. It has been reported that other mutations in this region cause myocardial dysfunction and/or fatal arrhythmia. We studied the functional characteristics of the K817E mutant channel, comparing the whole-cell currents in HEK293T cells expressing the wild-type or mutant channel under voltage clamp. The K817E mutation decreased the peak current density (142 pA/pF at a test potential of 0 mV) compared with the wild type channel (268 pA/pF at a test potential of -20 mV). The mutation also right-shifted the voltage-steady-state activation curve by ~21 mV and left-shift the voltage-steady-state inactivation curve by ~3 mV. The modulation of activation kinetics by the K817E mutation limits the availability of the Na^+ channel during an action potential, and this may underlie Brugada syndrome.

(COI: No)

P2-196

Crosstalk between mitochondria and sarcoplasmic reticulum in sinoatrial node cells

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We reported that the mitochondrial Na-Ca exchanger, NCLX, functionally couples with the sarcoplasmic reticulum (SR) Ca pump, SERCA, in B lymphocytes as well as in a cardiac cell line HL-1 (Kim et al., J Physiol, 2012; Takeuchi et al., Sci Rep, 2013; Takeuchi et al., J Physiol Sci, 2014). In the HL-1 cells, the Ca crosstalk between mitochondria and SR via NCLX modulates the automaticity. However, there is little information on the role of NCLX and NCLX-mediated mitochondria-SR crosstalk in the real pacemaker cells, sinoatrial (SA) node cells. In the present study, we performed electron microscopic observation and simulation analyses to examine the role of mitochondria-SR crosstalk in the SA node cells.

In the mouse atrial cells, mitochondria and SR occupied 17.25 ± 2.50 and 4.63 ± 0.78% of total cell area, respectively. On the other hand, it was 14.65 ± 1.40 and 3.86 ± 0.61% in the SA node cells, which were comparable with those in atrial cells. In both types of cells, a considerable fraction of SR localized adjacent to mitochondria. According to the geometry, we modified mathematical models of SA node cell. It was suggested that mitochondria-SR crosstalk affected the firing rate.

(COI: No)

P2-197

Characterization of the cardiovascular anomalies in the *Foxc2* deficient mouse

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Foxc2 gene, one of the forkhead transcriptional factors, is expressed in several embryonic tissues. We found the severity of cardiovascular anomalies in *Foxc2* deficient (*Foxc2-/-*) fetuses had a wide spectrum depending on their backgrounds. In this study, we observed 129 x Swiss Black-*Foxc2-/-* embryos (Winnier et al., 1997) at embryonic day 10.5 and characterized the pharyngeal anomalies. The combining approaches using: Intracardiac ink injection, and serial sections with hematoxylin-eosin staining were performed for the morphological analysis of pharyngeal arches. B6-*Foxc2-LacZ* knock-in embryos at E10.5 were also examined for the gene expression. *Foxc2-/-* embryos showed aplasia of the 4th pharyngeal arch arteries (PAAs), and deformities of the 4th pharyngeal pouch. *Foxc2-LacZ* embryos showed X-gal positive mesoderm cells including PAA endothelia. These data indicate that cardiovascular anomalies in 129xSwiss Black- *Foxc2-/-* mice are due to not only simple aplasia of PAAs and deform of the pharyngeal pouch but also other additional factors which would be concerned.

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(COI: No)

P2-198

Donepezil, Acetylcholinesterase Inhibitor, Attenuates LPS-induced Inflammatory Response in Murine Macrophage RAW 264.7 through Inhibition of NF-κB Translocation

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Introduction: We have previously demonstrated that pharmacotherapy with donepezil suppresses post infarct cardiac remodeling in a murine model of ischemic heart failure. However the precise mechanism is still unknown. Because inflammation is a pathological key event in the cardiac remodeling, we investigated the hypothesis that donepezil acts as an inhibitor of inflammatory mediators.

Methods and Results: RAW 264.7 cells were pretreated with donepezil prior to a pro-inflammatory stimulation by lipopolysaccharide (LPS). Donepezil significantly reduced intra- and extracellular levels of various pro-inflammatory mediators and attenuated nuclear translocation of nuclear factor-kappa B (NF-κB), indicating that donepezil showed anti-inflammatory effect. Acetylcholine (ACh) failed to inhibit the LPS-induced cellular responses. Moreover, ACh receptor blockers were ineffective in the anti-inflammatory effect of donepezil. Other kinds of acetylcholinesterase inhibitors did not show the anti-inflammatory properties. These results suggest that a non-neuronal cholinergic system would not be involved in the donepezil-induced signaling pathway and that the anti-inflammatory effect of donepezil would be independent of its acetylcholinesterase inhibition.

Conclusion: Donepezil attenuates pro-inflammatory response by inhibiting NF-κB activation, thereby may contribute to the cardioprotection during the post infarct cardiac remodeling process.

(COI: No)

P2-199

Effect of aging on the increase in concentration of oxygenated hemoglobin in the prefrontal cortex associated with the Stroop interference task

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We have examined the effect of aging on cognitive function tested with Stroop color-word test (SCWT), which was performed in young (n=9, 23 ± 1 yrs) and elderly (n=9, 64 ± 1 yrs) subjects. The subjects were instructed to answer the displayed color of an incongruent color word. The total time period and the number of errors during SCWT (100 trials) were defined as index of cognitive function. The concentrations of the oxygenated-hemoglobin (Oxy-Hb) were simultaneously measured in 22 sites of the prefrontal brain with a multichannel near-infrared spectroscopy (NIRS) to monitor regional cerebral blood flow (rCBF). The total time period for SCWT was longer in elderly (95± 5 s) than young subject (64± 5 s), and the number of errors tended to be greater (elderly 3.2± 0.6 vs. young 1.7± 0.7). The Oxy-Hb in the dorsolateral prefrontal and lateral frontopolar cortices (Broadmanns areas 46 and 10) increased during SCWT in both groups. However, the magnitude of the Oxy-Hb increase in elderly (0.04-0.06 μM*cm) were less than that of young subjects (0.07-0.10 μM*cm). Furthermore, the Oxy-Hb gradually increased and peaked at the later period of SCWT in elderly, while the Oxy-Hb abruptly increased in young subjects as soon as SCWT was started. These results suggest that the attenuated response in rCBF of the prefrontal cortex in elderly subjects is in good association with the age-related cognitive decline.

(COI: No)

P2-200

Changes of the cardiovascular parameters during 90°Head-up tilt in aging rats

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To elucidate the changes of the cardiovascular parameters in response to the 90° Head-up tilt(HUT) for 30 min in anesthetized aging rats, we measured systemic arterial blood pressure(BP), blood flow in common carotid artery(BF) and heart rate(HR) by analog-digital device(MP36; Biopac, USA). Under anesthesia(urethane, 1.0-1.5 g/kg, ip), we inserted a BP catheter into a right common carotid artery toward the heart, attached a BF probe to an artery(ultrasound flowmeter T206;Transonic, USA), and placed ECG electrodes on a subcutaneous for HR counting. In aging rats, after onset of HUT posture the BP and BF immediately decreased to 73.2 ± 11.8 mmHg and 4.3 ± 0.23 ml/min at 9.1 ± 6.0 sec, respectively, from each value before HUT (83.3 ± 12.4 mmHg, 4.7 ± 0.32 ml/min; n=5, $p<0.05$). On both of the traces, BP and BF turned to values under supine before HUT, nevertheless it spent a lot of time for becoming to the steady state, and the HR gradually increased after HUT;There was statistically difference vs. control at 30 min. These results indicated that initial changes in BP and BF were caused by the hydrostatic pressure gradient as same as adult rats. From the viewpoint of these changes throughout HUT, the aging will lead a smaller compliance of artery and a weaker vaso-constriction, leading the possibility that the baroreflex works inadequately in aging rats, and increase in HR compensate for the smaller vascular resistance to maintain BP.

(COI: No)

P2-201

Sex differences in heart rate variability and circulation in young and elderly volunteers after postural change

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Little is known about the influence of an individual's sex on heart rate variability (HRV) and circulation in various recumbent positions.

The purpose of this study was to evaluate whether there are differences in HRV and circulation at three positions (supine, right lateral, and left lateral decubitus positions) between male and female adults.

We recorded electrocardiograms and measured blood pressure (BP) in the three positions for 10 min in 58 young (23.0 ± 0.6 years) and 50 elderly (74.1 ± 0.8 years) volunteers. For the young group, no significant sex differences were observed for heart rate (HR) or high frequency (HF) components in the supine position. Both systolic BP and the ratio of low frequency to HF components (LF/HF) were significantly higher for men than for women. Although HR significantly decreased after changing position from the supine to the left recumbent position in both sexes, HF components and LF/HF ratios did not change. This phenomenon was also observed in the elderly group. For the elderly group, HR and HF components showed no differences between men and women in the supine position, but the LF/HF ratio was higher in men.

These results suggest that postural change to the left recumbent position has no influence on autonomic nervous activity. Additionally, HR reduction in the left recumbent position occurred equally in men and women, regardless of age.

(COI: No)

P2-202

AT1 promotes oxLDL-induced cell responses through interact with LOX-1

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Objective: LOX-1-mediated actions by oxidized low-density lipoprotein (oxLDL) play a critical role in atherogenesis. Angiotensin II type 1 receptor (AT1) is involved in atherosclerotic development aside from regulating blood pressure. Here, we investigated direct interaction of these two receptors and its role in vascular dysfunction.

Methods and Results: LOX-1 was found to form a complex with AT1 in plasma membrane by a co-immunoprecipitation assay and an in situ proximity ligation assay. Additional expression of AT1 promoted LOX-1-mediated cell responses such as ERK phosphorylation, G-protein activation, and NF-κ B and SRF activation in CHO cells expressing LOX-1 and AT-1 or HUVEC. We also found that AT1 blocker (ARB), olmesartan, suppressed oxLDL-induced ERK phosphorylation in HUVEC. In vivo, SHRSP were given high fat diet and olmesartan (0.1 mg/kg/day) or hydralazine (2 mg/kg/day) for a week from the age of 8 weeks. Arterial lipid accumulation in SHRSP, where LOX-1 is known to be involved, was decreased by ARB-treatment irrespective of blood pressure compared with hydralazine-treatment ($p<0.05$). There was no noticeable difference in plasma total cholesterol, HDL cholesterol, triglyceride, and fatty free acids between two groups.

Conclusion: Interaction between AT1 and LOX-1 might promote modified LDL-mediated vascular reactions.

(COI: No)

P2-203

Critical role of human hepatoblastoma stem cells in tumor angiogenesis

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Tumor angiogenesis play pivotal roles in tumor development, progression and metastasis. Endothelial cells (ECs) in the tumor vasculature are traditionally thought to be derived from normal ECs in existing blood vessels near the tumor. However, Wang et al. and Ricci-Vitiani et al. reported that CD133 positive cancer stem cells (CSCs) in glioblastoma generate tumor endothelial progenitor cells, which further differentiate into tumor endothelial cells. Therefore, we examined the relation between CSCs and tumor angiogenesis in association with CD133, which is a marker of CSCs.

Human hepatoblastoma cells (HuH-6 Clone-5) were cultivated and the fraction of side population (SP) cells was analyzed with flow cytometer. The SP cells were injected subcutaneously into NOD/SCID mice. The digested xenograft tumor fragments were cultured and the tumor sphere assay was performed. The spheres were cultivated using 3D collagen gel culture methods.

Some spheres formed CD133 positive capillary-like structures. TEM images of them confirmed the structures with identifiable lumens. These observations suggest that CD133 positive CSCs in hepatoblastoma differentiate into tumor endothelial cells. The hypoxia inducible factor-1 α (HIF-1 α) has been regarded as the most important transcriptional factor promoting tumor angiogenesis by up-regulating pro-angiogenic genes such as vascular endothelial growth factor (VEGF). We also discuss the relationship between HIF-1 α and VEGF.

(COI: No)

P2-204

Real-time intracellular Ca^{2+} imaging in the mouse heart *in vivo*

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Ca^{2+} imaging in cardiomyocytes is an effective means for analyzing diastolic and systolic states of the heart. We have previously reported spontaneous Ca^{2+} waves / transients in the isolated perfused mouse heart by using Ca^{2+} indicators (e.g., 58th Annual Meeting of Biophysical Society, USA, 2014). In the present study, we attempted to develop a method for real-time imaging of intracellular Ca^{2+} in the beating mouse heart *in vivo*. Namely, the anesthetized open-chest mouse under ventilation was placed on a custom-made microscope stage (25 cm × 35 cm; Olympus). Then, Ca^{2+} indicators were injected into the left ventricle through the apex of the heart, and perfused in the coronary system *in vivo*. Changes in $[Ca^{2+}]i$ were detected by using the microscope equipped with a spinning disk confocal unit (CSU 21, Yokogawa). EMCCD camera (iXon, Andor) was used for signal detection. Ca^{2+} waves were observed in cardiomyocytes *in vivo* when the body temperature of the mouse was lowered. The present system has a wide range of application potentialities, and it will be useful in future studies in wide-ranging fields of cardiovascular physiology.

(COI: No)

P2-205

Regulation of store-operated Ca^{2+} influx by STIM1 phosphorylation

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Background: STIM1 is an endoplasmic Ca^{2+} sensor, which plays a critical role in triggering the store-operated Ca^{2+} influx (SOC). The present study elucidates the role of phosphorylation of STIM1 in the regulation of SOC.
Methods: Porcine aortic endothelial cells (PAECs) and HeLa cells were used. The changes in [$CaCa^{2+}$]i was monitored with a front-surface Fura-2 fluorometry. The phosphorylation of STIM1 was evaluated with Phos-tag SDS-PAGE analysis. The mutants of STIM1 were used to determine phosphorylation sites and the functional significance of STIM1 phosphorylation.
Main results: Thapsigargin-induced depletion of Ca^{2+} store in the absence of extracellular Ca^{2+} caused a stoichiometric phosphorylation of STIM1 in both PAECs and HeLa cells. Subsequent replenishment of extracellular Ca^{2+} induced Ca^{2+} influx; however, the level of phosphorylation remained unchanged. Thrombin induced STIM1 phosphorylation in PAECs, which was reversed upon Ca^{2+} replenishment. ML-9 and wortmannin inhibited thapsigargin-induced STIM1 phosphorylation and sustained component of SOC. Analysis of the truncated mutants of STIM1 revealed the C-terminal 40 residues to contain the phosphorylation site. In HeLa cells expressing STIM1 with the Ala mutation of all four putative phosphorylation sites in this region, thapsigargin-induced Ca^{2+} influx was attenuated; however, the puncta formation of STIM1 was unaffected.
Conclusions: STIM1 is phosphorylated following store depletion. This phosphorylation supports the sustained phase of SOC.

(COI: No)

P2-206

Smooth muscle cells of venules show different responses to various transmitters, compared to those of arterioles

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It has been well known that shape and distributions of smooth muscle cells (SMC) are different between artery/arterioles and vein/venules. Very few data has been available on physiological characters of SMC of vein/venule. We observed [Ca2+]i dynamics of SMC of intact venules to clarify which transmitters can elicit any response of venules, and compered with those of SMC of arterioles. Venules and arterioles were isolated from rats, and loaded with a fluorescent Ca2+-indicator. Ringer solution containing various transmitters/ modulators was perfused around the specimens. SMC of arterioles are fusiform in shape, while those of venules are polygonal. 5-HT, ATP, and angiotensin II elicited an increase in [Ca2+]i in most SMC of arterioles. On the contrary, the [Ca2+]i increase in SMC of venules during 5-HT or ATP stimulation was faint, but NorAd can induce an evident increase in SMC. Response to angiotensin II was also significant. 5-HT and ATP has not been considered as "pressor substances", even though both induced strong vasoconstriction of arterioles. This may mean that not only vasoconstriction of arterioles is a unique factor for increase of systemic pressure. However substances known as "pressor substances" induce SMC response of venules as well as arterioles. The reduction of vascular volume of venous system may play a pivotal role in the increase of systemic blood pressure.

(COI: No)

P2-207

Localization and phosphorylation of focal adhesion kinase (FAK) in splenic sinus endothelial cells

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The endothelial cells that line sinus capillaries in the red pulp in the spleen are structurally different from other vascular endothelial cells. Although the unique structures of the sinus endothelial cells are believed to be formed for the passage of blood cells in the splenic cords surrounding the sinus endothelium, the way the passage of blood cells is controlled in the endothelium remains unclear. The cell-cell junctions of the sinus endothelial cells are constituted of poorly developed tight junctions and predominant adherens junctions. The cell-extracellular junctions is composed of vitronectin, one of extracellular matrices (ECM) and integrin $\alpha v \beta 5$ at focal adhesions, and moreover integrin $\alpha v \beta 5$ is localized not only ad focal adhesions but also in the entire circumference of the endothelial cells. Focal adhesions in endothelial cells are important mediating-sites interacting cytoskeletons and ECM via integrins and integrin-associated intracellular proteins to regulate variety of processes such as cell growth, cell shape changes, cell migration, differentiation, barrier regulation, and so on, and these processes are mediated by a non-receptor protein tyrosine kinase, FAK. Recently, in addition to the roles on cell-ECM signaling events, a role for FAK in regulating cell-cell junctions has come out. Therefore, immnolocalization of FAK and tyrosine-phosphorylated FAKs in the sinus endothelial cells of the rat spleen has been examined by immunofluorescence microscopy. Labeling for tyrosine-phosphorylated FAKs was present at the basal part and the cell-cell junctions of the endothelial cells.

(COI: No)

P2-208

The central pathway between the TMN and NTS increases arterial pressure

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The nucleus tractus solitarius (NTS) is one of the nuclei which receive histaminergic neurons from the tuberomammillary nucleus (TMN) in the posterior hypothalamus. However the physiological role of the TMN-NTS pathway remains unclear. We previously found that NTS histamine plays a role in regulating cardiovascular system via activation of histamine receptor H1. In this study, we investigated whether the TMN-NTS pathway is involved in the central cardiovascular regulation. We electrically stimulated the TMN and found pressor and tachycardiac responses. The pressor responses were partially inhibited by cetirizine, a H1 receptor antagonist, microinjected into the NTS whereas we failed to see the inhibitory effects on the heart rate responses. Moreover the TMN neurons were identified to directly project to the NTS by a retrograde tracer, Fluoro-Gold. Together with our previous reports, these findings further demonstrate that the TMN-NTS pathway is involved in the central pressor responses presumably under high arousal phase such as exercise.

(COI: No)

P2-209

Pathological analysis of atherosclerosis in human disease model ApoE-KO mouse

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Background and Aim: In Japan, atherosclerosis and the related-diseases are increasing with Westernized life style, and the death-related atherosclerosis occupies the higher rank of the cause of death. It is extremely crucial issue for translational research to understand the disease development process using mouse model for atherosclerosis.
Methods: In order to induce atherosclerosis, ApoE-KO mice were administered high-fat diet for 16 weeks, and these lesions were histopathologically examined (H&E and Elastica van Gieson stains).
Results: Many atherosclerosis similar to human lesions were seen, and the lesions were classified into 3 types: 1) early lesion, 2) progressive lesion and 3) combined lesion. Early lesion shows fatty streak which involves foamy cell accumulations (macrophages containing lipid). Progressive lesion is composed of foamy cell accumulations, fibrous cap and lipid-core. Combined lesion is determined with accompanying by calcification and stenosis.
Conclusions: Since atherosclerosis lesions in ApoE-KO mouse are histopathologically similar to humans, it is expected for evaluation of therapeutic efficiency and elucidation of the molecular mechanism.

(COI: No)

P2-210

Roles of CXCL17 and CXCL17-responding MDSCs in angiogenesis during tumor progression

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Recently, Chemokines have been regarded as important targets in cancer therapy, because they participate in tumor metastasis and recruitment of immune suppressor cells. Our previous studies showed that CXCL17 expression by tumor cells enhanced the tumor growth in mice, and demonstrated that CXCL17 also induced migration of myeloid-derived suppressor cells (MDSCs) into tumor sites. In the present study, we focused on tumor blood vessels as one of responsible factors in the progression of CXCL17-expressing tumors to clarify the effects of CXCL17 and CXCL17-responding MDSCs on tumor blood vessels at the same days after transplantation or in the same tumor size. CXCL17-expressing colon26 cells (a mouse colon cancer cell line) injected subcutaneously into BALB/c mice, showed an increase in CD31+ endothelial cell areas and well-maintained blood vessel meshworks compared with CXCL17-nonexpressing control tumors. Moreover, the number of pericytes increased in the tumor vessels of these CXCL17-expressing tumors. Furthermore, the CXCL17-responding MDSCs were separated by their chemotactic activity in the chemotaxis assay using mouse splenocytes. The transplantation of tumor cells together with CXCL17-responding MDSCs enhanced tumor angiogenesis compared with tumor cells transplantation with CXCL17-nonresponding cells. These results suggest that CXCL17 and CXCL17-responding MDSCs may be responsible for the tumor progression by inducing the structural and function changes of tumor blood vessels.

(COI: No)

P2-211

Microvesicles released from two mouse mammary carcinoma cell lines contain premature VEGF-C

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Enhanced expression of VEGF-C in tumor cells and subsequent elevation of lymphangiogenesis has been reported to promote lymphatic metastasis. Cell-derived microvesicles are endogenous carriers transporting proteins between cells. Using two mouse mammary cancer cell lines; BJMC338 cells with low metastatic propensity and BJMC3879 with higher metastatic propensity, we found that these two cell lines released microvesicles (MVs) containing premature VEGF-C (pre-VEGF-C) into the culture medium. Both cell lines released shedding vesicles (500-1,000nm) and exosomes (50-100nm) into media. Western blot analysis of VEGF-C demonstrated that exosomal expression of pre-VEGF-C was stronger than that of shedding MVs. Using mouse endothelial cell line (UV2) which express VEGFR3, tube formation assay demonstrated that the formed-tube area was increased when incubated with BJMC cells-derived MVs. To detect the processing of VEGF-C/VEGFR3 system, immunofluorescent analysis of phospho-VEGFR3, Akt and phospho-Akt in MV-treated UV2 cells were performed. UV2 uptook MVs of both BJMC cell lines, and then expressed phospho-VEGFR3 and phospho-Akt. These results indicated that both MVs of two cell lines enhanced lymphangiogenesis, while the malignancy is different between two BJMC cell lines. More studies are needed to show the target cells of pre-VEGF-C packed in MVs.

(COI: No)

P2-212

Inhibition of Cyclooxygenase Closes Chicken Ductus Arteriosus

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Background: Ductus arteriosus (DA) is an essential fetal artery that connects the main pulmonary artery and the descending aorta. Mammalian DA closes right after birth through vasoconstriction via decreases of circulating prostaglandin E_2 (PGE_2) transferred from placenta. Avian DA also closes after birth although avian has no placenta that is a source of PGE_2 in rodent and mammalian. Previous research demonstrated that PGE_2 signal pathway is not involved in constriction of isolated chicken DA. However, *in vivo* effects of PGE_2 in avian DA are little understood.
Aim: The aim of this study is to elucidate effects of PGE_2 in chicken DA closure.
Method and results: First, we measured blood concentration of PGE_2 in chicken at day 19 embryo by enzyme immunoassay. Blood concentration of PGE_2 in chicken was significantly higher than that of rat at day 21 embryo. Next, in chicken at day 19 embryo, Enzyme immunoassay revealed that PGE_2 in the DA was higher expressed than that of the Aorta. These data suggested that PGE_2 works on fetal chicken DA. Finally, we performed a rapid whole-body freezing method to evaluate DA closure *in vivo*. We measured internal diameter of DA at 2hrs after *in ovo* injection of indomethacin, which is a nonselective cyclooxygenase inhibitor. Indomethacin constricted DA at day 19 embryo *in vivo*, but did not constrict the Aorta. These data suggested that PGE_2 is an important factor in avian although avian has no placenta that is a source of PGE_2.
Conclusion: Inhibition of cyclooxygenase closes chicken DA. Prostaglandin E_2 signal may play an important role in an acute response of chicken DA closure.

(COI: No)

P2-213

Role of TRPC3 channels in cardiac fibrosis

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The cardiac pathological response to sustained pressure overload involves left ventricular hypertrophy and dysfunction along with interstitial fibrosis. Accumulating evidences indicate that activation of small GTP-binding protein RhoA plays a critical role in cardiac fibrosis. Here, we found that an inhibition of diacylglycerol-activated transient receptor potential canonical subfamily 3 (TRPC3) channels suppressed pressure overload-induced cardiac dysfunction and cardiac fibrosis via suppression of RhoA-dependent signaling in rodent hearts. Knockdown of TRPC3 completely suppressed fibrotic responses of rat cardiac fibroblasts induced by mechanical stretch and transforming growth factor (TGF) β. Collagen deposition and cardiac diastolic dysfunction were significantly suppressed in TRPC3-deficient mice compared with wild type (WT) mice. Inhibition of TRPC3 significantly reduced RhoA activity and expression levels of fibrotic genes in mouse hearts induced by pressure overload and rat cardiomyocytes induced by mechanical stretch and TGF β. A tubulin-binding Rho guanine nucleotide exchange factor, GEF-H1, was found to be up-regulated and activated in pressure-overloaed WT hearts. The up-regulation and activation of GEF-H1 were significantly suppressed in TRPC3-deficient hearts. These results strongly suggest that TRPC3-mediated cation influx contributes to pressure overload-induced cardiac fibrosis and dysfunction through mechanosensitive GEF-H1-mediated RhoA activation.

(COI: No)

P2-214

Knock-in mouse model of hypertrophic cardiomyopathy caused by troponin T mutation

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We created knock-in mice with a missense mutation S179F in cardiac troponin T (cTnT), which had been found to be associated with human hypertrophic cardiomyopathy (HCM). Membrane-permeabilized cardiac muscle fibers from these mice showed significantly higher Ca^{2+} sensitivity in force generation than those from wild-type mice, while the maximum force-generating capabilities being not different from wild-type mice. This demonstrated that the mutation S179F does have a Ca^{2+} sensitizing effect *in vivo* on force generation in cardiac muscle, consistent with previous *in vitro* reconstitution studies on other HCM-causing cTnT mutants. The knock-in mice suffered from sudden death frequently, and histological examination of cardiac sections showed significant displacement fibrosis, myocardial hypertrophy, and myocyte disarray. Echocardiography showed that left ventricular (LV) end-diastolic dimension was significantly decreased with no changes in ejection fraction. In vivo cardiac catheter measurements showed a significant increase in $LVdP/dt_{min}$ with no changes in $LVdP/dt_{max}$. These results indicate that the knock-in mouse with S179F mutation in cTnT is a useful mouse model of HCM closely recapitulating the clinical phenotypes of human patients.

(COI: No)

P2-215

Survival benefit of ghrelin in the heart failure due to dilated cardiomyopathy

Zhan, Dongyun[1]; Du, Chengkun[1]; Morimoto, Sachio[2]; Akiyama, Tsuyoshi[1]; Schenke, Daryl[3]; Hosoda, Hiroshi[1]; Kangawa, Kenji[1]; Shirai, Mikiyasu[1] (*[1]Natl. Cereb. Cardiovas. Ctr., Suita, Japan.; [2]Fac. Med. Sci., Kyushu Univ., Fukuoka, Japan.; [3]Otago Univ., Otago, New Zealand*)

Although ghrelin has been demonstrated to improve cardiac function in heart failure, its therapeutic efficacy on the life expectancy remains unknown. We aim to examine whether ghrelin can improve the life survival in heart failure using a mouse model of inherited dilated cardiomyopathy (DCM) caused by a deletion mutation δK210 in cardiac troponin T. From 30 days of age, ghrelin (150 μg/kg/day) was administered subcutaneously to DCM mice, control mice received saline only. The survival rates were compared between the two groups. After 30-day treatment, functional and morphological measurements were conducted. Ghrelin-treated DCM mice had significantly prolonged life spans compared with control DCM mice. Echocardiography showed that ghrelin reduced left ventricular (LV) end-diastolic dimensions and increased LV ejection fraction. Moreover, histoanatomical data revealed that ghrelin decreased the heart-to-body weight ratio, prevented cardiac remodeling and fibrosis, and markedly decreased the expression of brain natriuretic peptide. Telemetry recording and heart rate variability analysis showed that ghrelin suppressed the excessive cardiac sympathetic nerve activity (CSNA) and recovered the cardiac parasympathetic nerve activity. These results suggest that ghrelin has therapeutic benefits for survival as well as for the cardiac function and remodeling in heart failure probably through suppression of CSNA and recovery of cardiac parasympathetic nerve activity.

(COI: No)

P2-216

The cardiac baroreflex sensitivity decreases depending on the intensity and duration of treadmill exercise in cats

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We have reported that central command induces selective inhibition for the cardiomotor limb of the aortic-baroreceptor reflex at the onset of spontaneous motor activity in decerebrate cats (Matsukawa et al. Am J Physiol Heart Circ Physiol 303: H464-474, 2012; Auton Neurosci 179: 75-83, 2013). In this study, we examined whether central command also inhibits the sensitivity of cardiac baroreflex during treadmill exercise for one min (walking speed, 20-70 m/min) in 3 conscious cats. The baroreflex bradycardia response was elicited by brief occlusion of the abdominal aorta repeatedly given before, during (at the onset, 15 s, 30 s, and 45 s) and after exercise. The baroreflex sensitivity (BRS) was estimated from the slope of the mean arterial pressure-heart rate curve. During exercise at 20-40 m/min, the BRS was temporarily decreased at the onset of exercise alone but was maintained throughout exercise. On the other hand, the BRS was decreased at the onset of and during the later period (30-45 s) of exercise at a higher speed of 50-60 m/min. The characteristics of the changes in BRS were similarly observed following denervation of the carotid sinus nerves. These results suggest that the sensitivity of cardiac baroreflex is blunted at the onset and during the later period of treadmill exercise at a higher intensity, probably due to central command.

(COI: No)

P2-217 (AP-7)

Rapid cholinergic and delayed β-adrenergic vasodilatation in non-contracting muscles during one-armed cranking

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We have reported that the rapid cholinergic and delayed β-adrenergic vasodilatation increases blood flow of non-contracting vastus lateralis (VL) muscle during one-legged cycling (Ishii et al. 2013, 2014). It was unclear whether such mechanisms contribute to vasodilatation in non-contracting muscles during one-armed exercise. We examined the influences of atropine and/or propranolol on the blood flow responses of the contralateral biceps and triceps brachii and forearm extensor muscles and VL muscle during moderate one-armed cranking for 1 min (n=7). As an index of muscle tissue blood flow, relative concentration in oxygenated-hemoglobin (Oxy-Hb) was measured using near-infrared spectroscopy. The Oxy-Hb of the muscles increased during one-armed cranking. The increase in Oxy-Hb at the early period of exercise was blunted by atropine, whereas propranolol attenuated the later increase in Oxy-Hb during the exercise. Following combined atropine and propranolol, the Oxy-Hb decreased during the exercise. The influences of the autonomic blockades on the Oxy-Hb response were not different among the muscles. It was concluded that the rapid cholinergic and delayed β-adrenergic vasodilatation increased the blood flows of non-contracting arm and leg muscles during one-armed exercise.

(COI: No)

P2-218

Blockade of glycinergic inputs into the RVLM neurons enhances respiratory modulation of the cardiovascular sympathetic nerve in the in situ arterially-perfused preparation of rats

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It has been known that neurons in the rostral ventrolateral medulla (RVLM neurons) generate activity of the cardiovascular sympathetic nerve (SNA), and receive respiratory modulation from the respiratory center. Recently, we have reported that respiratory-related GABAergic inputs into the RVLM neurons regulate respiratory activity of the SNA. However, it is still unclear whether the other inhibitory inputs, glycinergic inputs, into the RVLM neurons are also related with the respiratory modulation of the SNA. In this study, we evaluated effects of a blockade of glycinergic inputs into the RVLM neurons on respiratory modulation of the SNA in the in situ arterially perfused preparation of rats. We injected a glycine receptor antagonist, strychnine (5 μM, 50 nL), into the RVLM bilaterally, and analyzed the effect on respiratory modulation of the SNA by the phrenic nerve activity-triggered average of the SNA. As a result, blockade of glycinergic inputs into the RVLM neurons elevated the basal SNA and enhanced the respiratory related SNA in the inspiratory phase. This data may indicate that glycinergic inputs into the RVLM neurons tonically inhibits the SNA, and also attenuates the respiratory modulation of the SNA.

(COI: No)

P2-219

Importance of body temperature regulation for the evaluation of cardiac function in anesthetized mice

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Cardiac function is an important determinant of circulatory homeostasis. Although recent technological development enables us to measure it easily and quickly even in small animals, there is considerable variability even in the same parameters across studies, presumably related to the experimental condition, e.g. body temperature. Since little is known how the cardiac function is affected by even relatively small changes in body temperature, we examined the effect of a small change in body temperature on the cardiac function in anesthetized mice. Male C57BL6 mice were anesthetized with isoflurane, intubated and mechanically ventilated. Left ventricular pressure (LVP) was measured by a miniature fiber optic pressure sensor (FISO-LS-PT9: tip diameter 0.9Fr, FISO Technologies Inc.) via the right common carotid artery. The first derivative of LVP (±dP/dt), heart rate and time constant of ventricular pressure decay (tau) were calculated electronically. Rectal temperature was continuously monitored and fluctuated between 35 and 38 ℃ by an automatic thermo control system (Thermo Plate, Tokai Hit). All measured parameters were strongly correlated with rectal temperature. The temperature-dependent changes in these parameters were attenuated by β1-adrenergic blockade with atenolol. In conclusion, precise control of body temperature is needed for the highly accurate and reproducible evaluation of cardiac function in anesthetized mice.

(COI: No)

P2-220 (AP-5)

In vivo assessment of cardiac autonomic nerve activities and identification of cardioprotective agents for heart failure treatment using atrial microdialysis technique

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Introduction: Sympathoexcitation and vagal withdrawal are causes of heart failure progression. Therefore, sympatho-suppression using beta-blockers has been a gold standard treatment for heart failure. We developed the atrial microdialysis technique to simultaneously assess cardiac sympathetic and vagal activities. Using this technique, we examined the effects of various pharmacological agents on cardiac autonomic nerve activities to identify cardioprotective agents.
Methods: In anesthetized rabbits, a dialysis probe was implanted into the right atrial myocardium near the sinoatrial node and was perfused by the Ringer's solution. Dialysate norepinephrine (NE) and acetylcholine (ACh) concentrations were analyzed as indices of cardiac autonomic nerve activities using high-performance liquid chromatography.
Results: 1) Electrical stimulation of sympathetic nerve or vagal nerve significantly increased dialysate NE or ACh concentration in a frequency-dependent manner. 2) Intravenous injection of medetomidine or guanfacine significantly increased dialysate ACh concentration. Furthermore, medetomidine significantly suppressed sympathetic NE release. 3) Intracerebroventricular injection of ghrelin significantly enhanced vagal ACh release to the heart.
Conclusions: Atrial microdialysis technique enabled us to simultaneously monitor cardiac sympathetic and vagal nerve activities. This technique may be useful for the identification of cardioprotective agents.

(COI: No)

P2-221

Cardiac interstitial small cells co-expressing prion protein (PrP) and cardiac troponin T (cTnT) spontaneously develop into beating atypically-shaped cardiomyocytes (ACMs)

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Atypically-shaped cardiomyocytes (ACMs) is a recently identified novel subpopulation of spontaneously beating heart cells found in the cultures of cardiac myocyte-removed crude fraction cells obtained from adult mouse cardiac ventricles. Most of ACMs are multinucleated and respond to β-adrenergic stimulation on the spontaneous Ca^{2+} transients. In the present study, we demonstrate the efficacy of cellular prion protein (PrP) as a surface marker of ACMs. PrP has been recently reported to serve as a surface marker for isolating cardiomyogenic progenitors from murine embryonic stem cells. Cells expressing PrP at the plasma membrane in the culture of the crude fraction cells were found to develop into beating ACMs by themselves or fuse with each other to become larger multinuclear beating ACMs. Combining PrP with a cardiac-specific contractile protein cardiac troponin T (cTnT) allowed us to identify native ACMs in the mouse cardiac ventricles as either clustered or solitary cells. The results suggest that the PrP- and cTnT-co-expressing cells identified in the mouse heart are native ACMs and their fusion results in the development of multinuclear beating ACMs, while there are some possibilities that the multinuclear cells due to the nuclear division in the ACMs. PrP- and cTnT-marked cells were also found in the interstitial spaces among ventricular myoycytes of adult human hearts, which suggests that the native ACMs exhibit life-long survival in the cardiac ventricles of both mice and humans.

(COI: No)

P2-222

Development of new leukocyte removal column aimed at suppression of the inflammatory response during cardiopulmonary bypass -Biological evaluation in a rat model-

Fujii, Yutaka; Shirai, Mikiyasu; Takewa, Yoshiaki; Tatsumi, Eisuke (*Natl. Cereb. Cardiovas. Ctr, Suita, Japan*)

Extracorporeal life support devices, such as the cardiopulmonary bypass (CPB), preserve the patient's life by providing adequate oxygen supply and blood flow to vital organs. However, previous studies have suggested that the interaction of blood and large artificial surface induces inflammatory response during CPB. As a result of series of chain reactions, the numerous powerful inflammatory mediators, including hormones and autacoids, are formed and released. Therefore, we developed the new leukocyte removal column (LRC) for attenuating the systemic inflammatory response during CPB. Rats were divided into the CPB group and the CPB with LRC group. CPB pump flow was maintained at 80 ml/kg/min. Blood samples were collected before (baseline), and 60 min and 120 min after initiation of CPB. We measured the differential count of leukocytes, pro-inflammatory markers such as (TNF-α) and biochemical markers (LDH, ALT, AST). Moreover, we also measured the wet-to-dry weight (W/D) ratio of the lung 120 min after the initiation of CPB. The increased levels of granulocyte count and pro-inflammatory cytokines in the CPB with LRC group were significantly attenuated than those in the CBP group(TNF-α :CPB group vs CPB with LRC group: 1510±121pg/ml vs 1112±160pg/ml). In addition, the level of W/D ratio was lower in the CPB with LRC group than in the CPB group. The data suggest that the new leukocyte removal column is useful for reducing the inflammatory response and lung edema during CPB.

(COI: No)

P2-223

Functional relationships between L-type $Ca_V1.3$ channel and the sustained inward Na^+ current in cardiac pacemaker cells

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The sustained inward Na^+ current (I_{st}) has been suggested to play a crucial role in pacemaker activity in sinoatrial node cells, although the molecular mechanism underlying this current remains unknown. We have recently found that genetic ablation of L-type $Ca_V1.3$ channel resulted in significant decrease in the low voltage-activated component of L-type Ca^{2+} current ($I_{Ca,\ L}$) as well as nearly complete disappearance of Ist in mouse sinoatrial node cells, indicating that $Ca_V1.3$ is responsible for at least two different current systems carried by Ca^{2+} and/or Na+ during the slow diastolic depolarization. We performed comprehensive analysis of $Ca_V1.3$ transcripts in rat sinoatrial node using second generation sequencing and single-cell PCR, yet no plausible splicing and editing sites remain to be identified in Ca^{2+}-selectivity filter. On the other hand, our patch-clamp recordings of recombinant $Ca_V1.3$ channel heterologously expressed in HEK cells revealed the multi-ion characteristics of the pore under different ionic environments for Na^+ and Ca^{2+}. Based on our experimental data, we will discuss the possibility that I_{st} can be mediated by $Ca_V1.3$ channel without an alteration in the molecular machinery of ionic permeation.

(COI: No)

P2-224

Luminescence imaging of HCN4 expression and phenotypic analysis of HCN4 TET-off mouse

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Among four subtypes of hyperpolarization-activated cyclic nucleotide-gated (HCN1~4) channel, HCN4 is major subtype in sino-atrial node (SAN). It has been reported that homozygous HCN4 knock-out mouse is embryonic lethal. In order to overcome this problem, we generated double knock-in mouse (HCN4Luc/tetA_TRE) that enables visualization of the locus of HCN4 expression with luciferase luminescence, and complete knockdown of HCN4 expression with doxyocycline (TET-off). We could successfully visualize pacemaker cells in vitro and ex vivo. The heart rate of TET-off mouse was significantly lower than WT; intermittent sinus pause or irregular RR interval was also observed. However, the heart rate of TET-off mouse was increased after the intraperitoneal injection of isoproterenol, and was not significantly different from that of WT measured in the same condition. We next compared parasympathetic response of TET-off and WT. For this purpose, we electrically stimulated right cervical vagal nerve. In WT, the heart rate was reduced in reversible manner. In contrast, vagal nerve stimulation in TET-off mice caused complete sinus pause, and recovery from sinus pause was significantly delayed after the termination of nerve stimulation. These finding suggested that HCN4 acted as a limiter for bradicardiac response, stabilizing spontaneous firing of SAN during parasympathetic stimulation.

(COI: No)

P2-225

Novel fish-derived peptide fragments which induce endothelium-dependent and -independent vasorelaxation

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Endothelium-dependent vasorelaxation (EDR) not only regulates physiological vascular tone but also counteracts abnormal vascular hypercontractions which lead to vasospasm and hypertension. Therefore, possible deterioration of these EDR functions of vascular endothelial cells under various pathological states such as aging, oxidative stress and lipid disorders, increases risk of vascular diseases, including heart attack and stroke. For the reliable prevention of such acute and lethal vascular diseases caused by endothelial dysfunctions, we attempted to discover foods or food components which help vascular endothelial cells induce EDR. After extensive screening, we eventually found out fish-derived peptide fragments (FDPFs) as the candidates. They were obtained by eatable enzyme digestion of fish proteins (bulbus arteriosus of skipjack tuna) and strongly induced both EDR and endothelium-independent vasorelaxation (EIR) of the porcine coronary arteries. Subsequently using tandem mass spectrometry and analysis software, Pep Novo, we identified four peptides in FDPFs and deduced other three peptides. All of these peptides are novel and previously unreported, and their synthetic peptides indeed induced both EDR and EIR of porcine coronary arteries. The mediation of NO in the EDR, at least in part, was evidenced by pharmacological studies with an inhibitor of eNOS and the fluorometric measurements of NO production and [Ca2+]i elevation in the endothelial cells.

(COI: Properly Declared)

P2-226

The novel role of calpain in SPC/Fyn/ROK pathway which mediates the signal transduction of abnormal vascular smooth muscle contraction

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Rho-kinase (ROK)-mediated Ca^{2+}-sensitization of vascular smooth muscle (VSM) plays a critical role for abnormal VSM contractions such as vasospasm. Previously we identified sphingosylphosphorylcholine (SPC)/Fyn/ROK pathway as a novel signaling pathway for abnormal VSM contraction. As possible downstream targets of Fyn tyrosine kinase, we identified vimentin by focused proteomics in which tyrosine-phosphorylated proteins were concentrated by anti-phosphotyrosine antibody 4G10 and identified by tandem mass spectrometry. Interestingly, western blot analysis revealed that SPC induced limited proteolysis of vimentin not only in human coronary artery smooth muscle cells (CASMCs) in culture, but also in vascular strips of the porcine coronary artery smooth muscle. Since vimentin is reported as the target of calpain, we examined the possible involvement of calpain. In CASMCs, SPC increased calpain activity, which was blocked by PD150606, a calpain inhibitor. Furthermore PD150606 inhibited the SPC-induced abnormal VSM contraction without affecting high K+-induced Ca2+-dependent contraction. These findings suggest the novel role of calpain in the signal transduction of abnormal VSM contraction.

(COI: No)

P2-227

Analysis of developmentally regulated cardiac titin isoform switch at birth

Hashimoto, Ken; Ujihara, Yoshihiro; Mohri, Satoshi (*First Dept Physiol, Kawasaki Med Sch, Kurashiki, Japan*)

During perinatal heart development, myocardial proteins often undergo isoform switch from fetal to neonatal/adult type, including myosin heavy chain and troponins. This is also observed in titin, a giant elastic protein in muscle sarcomere that critically defines a myocardial passive stiffness and thus ventricular filling in diastole. In mammalian heart, the long and compliant N2BA isoform (3.2-3.7 Mda) mainly expressed in fetus is dramatically replaced by relatively short and stiff N2B isoform (3.0 Mda) in neonate/adult, limiting the distensibility of neonatal/adult heart. Both isoforms are generated by alternative splicing from the single titin gene. Recently Rbm20 protein was identified as a main regulator of titin splicing, but its involvement in the perinatal titin switch is totally unknown. In mammals, the onset of air-breathing at birth leads to abrupt elevation of oxygen tension (PaO_2: 20mmHg in fetus to 100mmHg in adult). In this study, we focused on the involvement of Rbm20 in perinatal titin isoform switch and its possible regulation by elevated O_2 at birth. First we established the SDS-agarose gel electrophoresis system to detect mega dalton-sized N2BA/N2B isoform from mouse heart, and confirmed the perinatal isoform switch did occur. Exposure of fetal cardiomyocytes to atmospheric O_2 resulted in isoform shift from N2BA to N2B, suggesting that the elevated O_2 at birth is an upstream environmental cues that lead to titin isoform switch. We are now exploring the changes of Rbm20 expression and intracellular localization around birth, and its potential as an O_2-sensing molecule.

(COI: No)

P2-228

Electrical stimulation of the insular cortex increases regional blood flow in the mesencephalic ventral tegmental area in anesthetized rats

Liang, Nan; Matsukawa, Kanji; Ishii, Kei; Idesako, Mitsuhiro; Endo, Kana (*Dept Integr Physiol, Grad Sch Biomed Health Sci, Hiroshima Univ, Hiroshima, Japan*)

The mesencephalic ventral tegmental area (VTA) has been suggested to play a crucial role in the central cardiovascular control. In this pilot study, we aimed to clarify whether activation of the insular cortex (IC), a higher brain region that partially participates in the autonomic processing, modulates neural activity in the VTA as well as hemodynamics. In pentobarbital-anesthetized rats, catheters were inserted into the external jugular vein and carotid artery for the administration of drugs and measurement of arterial blood pressure, respectively. Activation of the right or left IC was evoked by an electrical stimulation (0.5-1.0 mA, pulse-width 1 ms, frequency 50 Hz) lasting for 4 or 10 s. A probe of laser Doppler flowmetry was inserted into the ipsilateral or contralateral VTA to measure regional blood flow. Electrical stimulation of the IC induced a pressor or depressor response in an intensity-dependent manner, while the regional blood flow in the ipsilateral VTA was increased consistently. The vascular conductance in the ipsilateral, but not contralateral, VTA was remarkable during the IC stimulation and much larger as compared to that without stimulation. The present results supported the anatomical evidence that connections exist reciprocally between the VTA and IC, and suggested that neural activity in the VTA is modulated by inputs from the ipsilateral IC.

(COI: No)

P2-229

Role of monoamine oxidase on hydroxyl radical production during ischemia/reperfusion in anesthetized rats

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Background: Excessive accumulation of monoamines exacerbates myocardial cell injury during myocardial ischemia/reperfusion. Meanwhile hydroxyl radical (OH) production by enzymatic degradation of monoamine has been suggested to exacerbate myocardial cell injury.
Purpose: To clarify the contribution of enzymatic degradation of monoamine by monoamine oxidase (MAO) to the ·OH production during myocardial ischemia/reperfusion.
Method: Using microdialysis technique with trapping reagent (4-hydroxybenzoic acid) in anesthetized rats, we monitored myocardial interstitial 3, 4-DHBA levels as an index of ·OH production during myocardial ischemia/reperfusion in the presence (pargyline group) and absence (control group) of MAO inhibitor, pargyline.
Result: In control group, dialysate 3, 4-DHBA concentration at baseline was 2.01 ± 0.26 nM. Dialysate 3, 4-DHBA concentration did not change during ischemia, but significantly increased to 3.80 ± 0.26 nM immediately after reperfusion and then kept this high levels by 60 min after reperfusion. In pargyline group, local administration of pargyline (1 mM) did not change dialysate 3, 4-DHBA concentration at baseline (1.88 ± 0.34 nM) and during ischemia, but significantly suppressed the increase in dialysate 3, 4DHBA after reperfusion.
Conclusion: MAO plays a significant role on hydroxyl radical production during ischemia/reperfusion, suggesting that MAO inhibition can attenuate cardiac ischemia/reperfusion injury.
(COI: No)

P2-230

Ghrelin acts directly on the central nervous system to suppress cardiac sympathetic tone and arrhythmias following acute myocardial infarction in rats

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Cardiac sympathetic nerve activity (CSNA) increases following acute myocardial infarction (MI). This increase adversely triggers life-threatening arrhythmias. Subcutaneous injection of ghrelin prevents the CSNA increase following MI and reduces the occurrence of arrhythmias, improving survival dramatically. The mechanisms by which ghrelin achieves this effect remains unclear. This study aimed to identify whether ghrelin acts directly within the brain to modulate CSNA following acute MI. Rats were anesthetized with urethane and surgically prepared: isolation and recording from cardiac sympathetic nerve, cannulation for systemic arterial pressure measurement, stereotaxic surgery for intracerebroventricular (icv) administering of ghrelin, ligation of the left anterior descending coronary artery (= MI). CSNA were continuously recorded prior to LAD occlusion, and for three consecutive hours following: no manipulation, MI + saline icv injection and MI + ghrelin icv injection. Within three hours of acute MI, untreated rats exhibited: a significant 200% increase in CSNA, a high incidence of arrhythmias and, thus a 34% mortality rate. The ghrelin injection reduced: the CSNA increase (62% increase), the incidence of arrhythmias and, thus, mortality rate (0% within three hours of MI). These results suggest that the direct action of ghrelin on the brain contributes to its suppressive effect on CSNA and arrhythmias following acute MI.
(COI: No)

P2-231

Properties of spontaneous activity in submucosal postcapillary venules of the rat stomach

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Background: We have recently reported spontaneous vasomotion (rhythmic constrictions) of the venules with diameters of 30-130 μm in the bladder, distal colon and stomach. Venular vasomotions may actively regulate tissue metabolite drainage. Here we further examined properties of spontaneous activity of postcapillary venules (PCVs) with diameters of 15-23 μm.
Methods: In the rat gastric submucosa, changes in PCV diameter, intracellular Ca^{2+} signalling of PCV mural cells and their morphology were examined by video imaging, Fluo-8 Ca^{2+} imaging and immuohistochemistry, respectively.
Results: PCV mural cells were α-smooth muscle actin-positive stellate cells and exhibited synchronous spontaneous Ca^{2+} transients associated with vasomotion. Inhibitors of endoplasmic reticulum Ca^{2+}-ATPase (10 μM CPA) or IP_3 receptor (100 μM 2-APB) abolished vasomotion. Nifedipine (1 μM) disrupted Ca^{2+} transient synchrony amongst PCV mural cells and prevented vasomotion. PCV endothelium expressed NO synthase, and tadalafil (1 μM), cGMP-specific phosphodiesterase type 5 (PDE5) inhibitor, abolished vasomotion, suggesting that NO may be continuously released from endothelium to produce cGMP in mural cells.
Conclusion: PCV in the rat stomach submucosa exhibits spontaneous vasomotion. The generation of spontaneous Ca^{2+} transients in PCV mural cells may depend on Ca^{2+} release/uptake of intracellular Ca^{2+} store, while voltage-dependent coupling relying on L-type Ca^{2+} channels appears to be essential for their synchrony. Continuous PDE5 activity that degrades cGMP may be crucial to maintain spontaneous vasomotion.
(COI: No)

P2-232

Relationship between local and overall pulse wave velocity in the aorta preserved well despite of extent and severity of atherosclerotic lesions in heritable hypercholesterolemic rabbits

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We investigated whether relationship between local pulse wave velocity (LPWV) and overall aortic pulse wave velocity (AoPWV) preserved or not despite of extent and severity of atherosclerotic lesions in Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbits aged 10-12 and 22-24 months. A catheter-tip transducer was indwelt in the ascending aorta (AA) and another catheter with three pressure sensors at the tip (40 mm intervals) was advanced to the distal end of the aortic arch (Position 0: P.0) under pentobarbital anesthesia (30 mg/kg, i.v.). Pressure waves were recorded at P.0, proximal (P.1), middle (P.2) and distal (P.3) thoracic aortas and proximal (P.4), middle (P.5) and distal (P.6) abdominal aortas simultaneously with those at AA by moving the catheter towards peripherals every 80 mm to calculate LPWV between adjacent aortic positions. There was little difference in averaged LPWV in different aortic segments (avgLPWV) and AoPWV in the normal and KHC rabbit groups aged 10-12 and 22-24 months. Strong positive correlation between avgLPWV and AoPWV was observed in the two strains of the two age groups. Mean error and standard deviation between avgLPWV and AoPWV were very small in Bland & Altman plots in the two strains of the two age groups. We can conclude that the relationship between avgLPWV and AoPWV preserved well regardless of extent and severity of atherosclerotic lesions.
(COI: No)

P2-233

Functional correlation of the arterial structure - a comparative study in the kidney and the skeletal muscle

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The differences of circulation in various organs are well known, but the structural differences are only poorly investigated. The wall structure of arteries was observed in the rat kidney and skeletal muscle. The wall thickness in the kidney was almost equal to that in the muscle about 40 μm in diameter, relatively thin in the larger arteries and thick in the smaller arteries. The smooth muscle cells were regularly arranged in parallel in circular or slightly spiral orientation, whereas those in the skeletal muscle were irregularly arranged in heterogeneous orientations. Extracellular matrices were more abundant in the arterial media in the skeletal muscle than in the kidney. The inner elastic lamina was continuous in the kidney, and longitudinal bundles in the skeletal muscle. The adventitial collagen fibers were abundant and dense in the skeletal muscle, and made scattering small bundles in the fluid filled spaces in the kidney. The arteries in the skeletal muscle were under severe mechanical stress in longitudinal direction during muscle contraction, and dilate dramatically by sympathetic stimuli. Those in the kidney keep the glomerular pressure constant and regulate it slightly. The differences of arterial structure in these organs reflect their functional differences well.
(COI: No)

P2-234

Comparison of heart rate, stroke volume, and blood pressure associated with acupuncture stimulation in supine and sitting subjects

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Acupuncture stimulation (ACP) induces the reduction of heart rate (HR) with the somato-autonomic reflex. Previous studies indicated that ACP responses were reflected by position change on HR. The present study was to investigate the comprehensive change of cardiovascular responses in supine and/or sitting position during ACP. Twenty-two healthy male volunteers participated in this examination. HR, stroke volume (SV), and blood pressure (BP) had been recorded on the both positions in same subjects. ACP had performed to left forearm for 60 seconds that inserted to a depth of 15 to 20 mm after approximately 15 minutes as the rest. Changes of cardiovascular response were compared between both positions. HR and diastolic BP (DBP) were significantly decreased that accompanied with the increase of systolic BP (SBP) and SV in supine position. In sitting position, the decrease of HR and the increase of SV, but not BP, were observed during ACP. Moreover, the decrease of HR in sitting position was stronger than that of supine, and the increase of SV in sitting was lower than that in supine. It seems that the decrease of HR is induced by ACP and increase of SV is dependent on the Starling's law. In sitting position, we are considering the relation of the gravitation and another factor to suppress the increase of SV during ACP.
(COI: No)

P2-235

Acupuncture stimulation induced-changes of hemodynamics

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Acupuncture (ACP) stimulation induces the significant reduction of heart rate (HR) via the somato-autonomic reflex. However, it is unknown whether there are influences on another hemodynamics by ACP stimulation. The present study was to assess ACP stimulation induced-comprehensive change of hemodynamics, which was HR, stroke volume (SV), and blood pressure (BP). Twenty-seven healthy male volunteers were participated in this examination. HR, SV, and BP were recorded at supine position. ACP, using stainless steel needle, was inserted to left forearm at a depth of 15 to 20 mm. ACP was continuously stimulated for 60 seconds after approximately 15 minutes as the rest. Each date were calculated the average value every 10 seconds, and were analyzed before, during and after ACP stimulation. HR had been decreased and SV had been increased throughout ACP stimulation. On the other hands, diastolic blood pressure was significantly decreased only the 20 seconds just after ACP inserting and recovered to control after 40 seconds. These results suggest that ACP stimulation induced-decreasing HR is mediated by somato-autonomic reflex and increasing SV is mediated by Starling's law of the heart. In contrast, although BP is affected by the ACP stimulation-induced somato-autonomic reflex partially, BP may recover to basic value immediately because of the baroreceptor reflex.

(COI: No)

P2-236

Analysis of rate-dependence of drug-induced QT-prolongation in human iPS-derived cardiomyocytes

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Human iPS cell-derived cardiomyocyte (hiPS-CM) is conceptually promising as an unlimited source of human cardiomyocytes for pre-clinical cardiac safety screening. However, the spontaneous activity of hiPS-CM impedes experimental manipulation of pacing rates, and therefore hinders the prediction of antiarrhythmic and proarrhythmic effects of rate-dependent drugs. In order to evaluate rate-dependent cardiac medicine in hiPS-CMs, we here developed a hiPS-CM model with matured phenotypes by transducing ventricular-specific gene X. The gene-X transduced hiPS-CMs ceased spontaneous beating both in single cells and multicellular sheets, but could generate ventricular-like action potentials when triggered by a stimulus and followed at various pacing rates (0.5, 1 and 2 Hz). Using this model, we could demonstrate quantitatively that E4031, a selective hERG blocker, prolongs durations of action potential in single myocytes and extracellular field potential in cardiac sheets, respectively. In the cardiac sheets, the technology enabled us to evaluate a reverse rate-dependence with or without E4031, and the addition of a selective I_{Ks} blocker, chromanol 293B, abolished it, showing a contribution of I_{Ks} to the reverse rate-dependence. Thus, our genetically modified hiPS-cardiomyocytes can be useful especially for evaluating of rate-dependent antiarrhythmic and proarrhythmic drugs.

(COI: No)

P2-237

Lymphatic circulation of cerebrospinal fluid in spinal regions - A morphological investigation and its clinical significance -

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Cerebrospinal fluid (CSF) is a fluid derived from choroid plexuses in the cerebral ventricles. It is generally considered that CSF is absorbed in several sites including venous sinuses via arachnoid villi, capillaries in the choroid plexus and lymphatic systems. Cranial drainage route along the olfactory nerve is one of the best known pathways of lymphatic absorbing systems whereas the regional lymphatic system at the spinal level remains obscure. We therefore aimed to elucidate the spinal CSF drainage route to the regional lymphatic system in Sprague Dawley (SD) rats using various CSF tracers. They were infused into the lateral ventricle with an osmotic pump followed by dissection of vertebral blocks, spinal nerves and regional lymph nodes. In addition, various types of cells of syngeneic rats were labeled with green fluorescent cell linker kit and injected into the cisterna magna. We found that only small tracers collected in lymphatics around the dorsal root ganglions, finally drained into the regional lymph nodes. Lymphoid cells also reached to each lymph node, whereas erythrocytes could not. Vital cells with chemotactic activity could migrate through this way though high-molecular-weight compounds could not pass alone. Hence, this drainage system might play an important role in the defense mechanism. It is highly indisputable that CSF in spinal subarachnoid space is absorbed in epidural lymphatics.

(COI: No)

P2-238

Role of TRPC3/TRPC6 activated by angiotensin II type 1 receptor in the slow force response to sustained stretch in mouse ventricular myocytes

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When cardiac muscle is stretched over several minutes, its intracellular Ca^{2+} transient and twitch force slowly increase, which is known as a slow force response to stretch (SFR). It has been reported that stretch-induced release of angiotensin II have been implicated in the SFR, to raise intracellular Na^+, followed by an increase in intracellular Ca^{2+} via Na^+/Ca^{2+} exchanger. However, the detailed pathways remain unclear. The activation of angiotensin II type 1 receptors (AT1R) has been reported to induce cation influx via TRPCs (TRPC3/TRPC6), which are known as nonselective cation channels. In this study, we tested the hypothesis that this pathway leads to SFR. A pair of piezo-positioned carbon fibers was attached to each end of an isolated mouse ventricular myocyte. The electrically stimulated cells were perfused in Tyrode solution at room temperature. Passive/active forces were calculated from carbon fibers bending. The stretch by moving carbon fibers led to an immediate increase in twitch force by Frank-Starling mechanism. The force slowly increased by 133.2 ± 2.0% of the force immediately for 300 s after the stretch. The SFR was blocked by AT1R inhibitor, olmesartan (97.6 ± 1.5%). 2-Aminoethyldiphenylborinate (TRPs inhibitor), YM-58483 (TRPCs inhibitor), and Rox-4560 (TRPC3/TRPC6 inhibitor) suppressed the SFR (93.2 ± 4.7%, 98.0 ± 2.4%, and 97.8 ± 2.4%). These results suggest that the activation of TRPC3/TRPC6 initiated by AT1R is involved in SFR.

(COI: No)

P2-239

Age-related effects of dexmedetomidine, an alpha-2 agonist, on coronary vasoactivity and ventricular contraction in guinea-pig hearts

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Dexmedetomidine (DEX) is a potent and selective alpha-2 agonist, and is widely used to produce both sedation, analgesia and anxiolytic effects. It has been reported that DEX sporadically causes bradycardia and hypotension, probably due to its sympatholytic activity. However, precise mechanisms such as involvement of peripheral postsynaptic alpha-2B receptors as well as direct effects on cardiac contractility are not yet clear. This study was carried out in order to evaluate the effects of DEX on coronary vasoactivity and ventricular contraction of guinea-pig hearts using Langendorff perfusion device. The heart was continuously perfused with Tyrode solution, and left ventricular pressure (LVP) and coronary perfusion pressure (CPP) were recorded using a PowerLab. DEX did not affect basal LVP, but markedly inhibited the increase of LVP induced by electro-field stimulation. The finding is consistent with a view that DEC acts on alpha2-receptors at sympathetic nerve terminals. On the other hand, we found that DEX also affected the coronary artery resistance, and that its effects altered with ages. Namely, DEX was without effect on CPP at ages of <4 weeks, but increased CPP at 4-8 weeks. The increase of CPP was more significant at 9-12 weeks, and was inhibited by prazosin, an alpha1/alpha2-adrenergic receptor antagonist. DEX may directly affect coronary vasoactivity via alpha1/alpha2-adrenergic receptors.

(COI: No)

P2-240

Spread of spontaneous transient hyperpolarizations within vascular endothelial cell layer

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The vascular endothelial cells are some 40 μm long and some 7 μm wide, and aligned with their major axes along the vessel. These cells are connected to each other with a lot of gap junctions and the whole endothelial layer is functioning as a syncytium. The electrical resistance seems to be larger in the circumferential pathway than in the axial one because the former includes more gap junctions than the latter. Using the conventional whole-cell clamp techniques, the spread of the spontaneous activities were examined in the endothelial layer acutely dispersed from the guinea-pig mesenteric artery. Two patch electrodes were applied to two individual cells separated either circumferentially or axially along the vessel and the membrane potentials (V_1 and V_2) were observed. The membrane potential was not constant but transient hyperpolarizations randomly occurred. The origin of such a spontaneous activity could be any cell within the syncytium. When one of the patched cells (V_1) is the origin, the amplitude of the hyperpolarization recorded from that cell is expected to be large and the amplitude ratio (V_2/V_1) should be smallest compared to any hyperpolarizations occur in cells other than patched ones. Among many recorded hyperpolarizations, large ones were selected and the distributions of the ratios (V_2/V_1) were compared between the circumferential and the axial directions. Unexpectedly the distributions were not so different between two directions and the electrical signals seem to spread within the endothelial layer into all directions rather equally.

(COI: No)

P2-241

Opening of Rat Ductus Arteriosus is promoted by inflammation

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Background: The ductus arteriosus(DA) is closing at birth. In premature babies, patent DA(PDA) can be fatal. We sometimes experience DAs reopen when the premature babies are exposed to the severe infection. The reopening of DA has not been cleared. We reported NFkB inhibition might facilitate DA closure. NFkB is one of transcriptional factors and relates to some inflammation. We thought NFkB works as an opening factor in the infection.
Methods and Results: We made LPS- stimulated rat models as the infectious models. LPS(100micro gram/kg) were injected to the maternal rats in pregnant day 18th and 19th. In the pregnant day 21st, the maternal rats were performed Caesarean sections under the anesthesia. Rat fetuses were injected IMD- 0354(NFkB inhibitor, IMD), carboxymethyl cellulose and PBS in intraperitoneal administration through maternal uterine wall. Rat fetuses were born and started breathing right after the infection. 30 minutes later, rat fetuses were sacrificed and frozen by liquid nitrogen. LPS injected rat fetuses DA tend to wider compared with nothing injected one. IMD injected after LPS injected rat fetuses DA tend to more narrow than other control models DA.
Conclusion: The present data demonstrated that LPS can open rat DAs and NFkB might facilitate DA opening.

(COI: No)

P2-242

Mechanism of negative inotropic effect on rat left ventricular in hyperthermia: role of TRPV1

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We previously reported that the effects of hyperthermia (42 ℃) on left ventricular (LV) mechanical work and energetics using the excised, cross-circulated rat heart model. We now investigated the effects of capsazepin (a TRPV1 antagonist) on LV mechanical work and energetics in hyperthermia. We analyzed the LV end-systolic pressure-volume relation (ESPVR) and the linear relation between the myocardial oxygen consumption per beat (VO_2) and systolic pressure-volume area (PVA; a total mechanical energy per beat) in isovolumically contracting rat hearts at 300-bpm pacing during infusion of capsazepin (50 μM) under hyperthermic conditions. Downward-shift of LV ESPVR from the control one was observed in hyperthermic-hearts, which was suppressed by capsazepin infusion. The mean slope and the mean VO_2 intercept, which is composed of each myocardial oxygen consumption for calcium handling in excitation-contraction coupling and for basal metabolism, of VO_2 -PVA relations were not significantly different in hyperthermic-hearts. The slope was unchanged but the VO_2 intercept was decreased in capsazepin treated-hyperthermic hearts. Protein levels of SERCA2 and phospholamban (PLB) were unchanged but phosphorylated PLB at threonine 17 was markedly decreased in hyperthermia. The latter decrease was not antagonized by treatment with capsazepin. These results suggested that negative inotropic effect in hyperthermic heart was, at least in part, mediated through TRPV1 signaling pathway.

(COI: No)

P2-243

Expression and functional significance of 5'-nucleotidase in lymphatic vessels

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The cellular expression and physiological function of 5'-nucleotidase (5'-Nase; CD73) in lymphatic vessels was investigated in rats by histochemical methods and in three-dimensional human cell culture system fabricated by layer by layer cell accumulation technique. Intense immunoreactivity of CD73 was predominantly found in the endothelial cells with cellular nuclei immunopositive for Prox1 (a master transcription factor of lymphatic endothelial cells) in specific regions of some large veins and lymphatics sprouting from those at mid-embryonic stage. The CD73-immunoreaction was thereafter demonstrated in several portions, especially in the leading tips, of developing lymphatics and every lymphatic of adult rat. The findings imply that 5'-Nase is preferentially expressed in lymphatic vessels both during vascular development and in mature state to operate on development and function of lymphatic and blood vessels. In addition, our knock down analysis using siRNA for 5'-Nase in 3D-human cell culture system showed an accelerated formation of lymphatic network, it suggesting an inhibitory action of 5'-Nase on control of lymphatic vascular development. In conclusion, it is likely that 5'-Nase regulates development and function of lymphatic vessels repressively in an autocrine fashion.

(COI: No)

P2-244

Glucagon-like peptide-1 (GLP-1) augments the stretch-induced release of atrial natriuretic peptide(ANP) from mouse atria

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Glucagon-like peptide-1 (GLP-1), a member of incretin peptides, has been shown to improve diabetes mellitus and heart failure. GLP-1 receptor is known to be linked with cAMP signaling pathway. Recent studies have shown that GLP-1 receptor is expressed in atrial myocytes. However, it has been controversial whether or not GLP-1 induces the release of atrial natriuretic peptide(ANP). This study was undertaken to clarify effects of GLP-1 on atrial function such as developed force and atrial rate, as well as the relationship between basal tension and ANP release.
Methods: Atria were excised from male mice (C57BL/6J) under deep anesthesia. We measured effects of GLP-1 on developed force, atrial rate, and ANP release from isolated atrial tissues under loads of various basal tensions (0.2-2.0 g).
Results & Discussion: The developed force of mouse atria was augmented depending on the basal tension, while atrial rate was rather decreased. GLP-1 (1 nM) caused significant increase of ANP release from isolated atria. GLP-1 tended to augment the basal tension-dependence of developed force. In sharp contrast, atrial rate was not affected by the application of GLP-1, although recent study has reported that GLP-1 is expressed in mouse sinoatrial node. These results suggest that GLP-1 augments the stretch-induced release of ANP from mouse atria, and that GLP-1 receptor signaling pathway may be linked with distinct regulatory mechanisms of atrial function.

(COI: No)

P2-245

The intracellular mechanisms of the constriction of the guinea pig hepatic veins

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We examined the intracellular mechanisms of the contraction on the guinea-pig hepatic veins. Phentolamin (3 μM) inhibited the contraction evoked by transmural nerve stimulation (TNS). 1 - 30 μM phenylephrine evoked vasoconstriction in a dose response manner. Nifedipine (1 μM) did not have any effects to the TNS-evoked contraction. CPA (10 μM) inhibited the contraction. In the presence of CPA, SKF-96365 (3 μM) increased the amplitude of the contraction. This enhancement effect of SKF-96365 was inhibited by N_ω-Nitro-L-arginine (10 μM). In contrast, the amplitude of contraction evoked by phenylephrine was decreased to only 70 % by 100 μM sodium nitroprusside. Y-27632 inhibited both the TNS- and phenylephrine-evoked contraction. The intensity of the intracellular Ca^{2+} indicator, Fluo-4, was increased in the smooth muscle cells when the cells were stimulated with 10 μM phenylephrine. These results suggest that the adrenergic nerves stimulate both the Ca^{2+} dependent and independent mechanisms to evoke the contraction in the smooth muscle cells of the hepatic vein. The L-type Ca^{2+} channels or the store operated Ca^{2+} channels do not seem to be the main source to cause the intracellular Ca^{2+} increase by the α adrenergic stimulation. The endothelial regulation of the vasoconstriction was limited.

(COI: No)

P2-246

Low birth weight is a predictor of later hypertension risk for both Japanese and Mongolian healthy young adults

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Low birth weight (LBW) was confirmed as a risk of high blood pressure (BP) in later stages of life (Barker DJ et al, 1989). Low-grade inflammation and deterioration of autonomic regulation play an important role in hypertension. However, the associations with birth weight are poorly understood. We examined these relationships in Mongolian and Japanese young adults and investigated whether ethnicity affected these relationships. We measured BP and heart rate variability at rest and during postural change from a supine to a sitting position in 21 Japanese and 16 Mongolian healthy volunteers aged 18-34 years. Blood cell counts, and total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol, triglyceride, and high sensitivity C-reactive protein levels were measured. Mongolians had lower levels of HDL-C than did the Japanese ($p < 0.01$). In Mongolians, the platelet count was higher in the LBW group than in the normal birth weight (NBW) group ($p < 0.05$). Following postural change, systolic blood pressure and heart rate showed no significant increases in the Mongolian and Japanese LBW groups, whereas the NBW groups had normal responses ($p < 0.05$). The Mongolian LBW group displayed a slight decrease in sympathetic nerve activity from a supine to a sitting position, although it increased in the Japanese LBW group ($p < 0.05$). We suggest that LBW is a predictor of later hypertension risks in both Japanese and Mongolian healthy young adults.

(COI: No)

P2-247

Differences in respiratory parameters between controlled $PetCO_2$ and controlled respiratory rate during cycling exercise

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Previous studies have reported that in oxygen uptake kinetics, the time constant increased during transition to heavy cycling exercise in respiratory alkalosis by a controlled end-tidal partial pressure of CO_2 ($PetCO_2$), while the time constant decreased in respiratory alkalosis by a controlled respiratory rate. This study aimed to clarify differences in respiratory parameters between a controlled $PetCO_2$ and a controlled respiratory rate. After $PetCO_2$ was controlled at 20 mmHg (PE) or the respiratory rate at 60 breath/min (RE) for 5 min, a subject performed baseline cycling exercise for 4 min at 10 W, followed by heavy cycling exercise for 6 min at the anaerobic threshold (AT) plus 40% of the difference between the AT and peak pulmonary oxygen uptake intensity. Throughout the experiment, $PetCO_2$ or respiratory rate was maintained at 20 mmHg or 60 breath/min, respectively. In the control experiment (CE), a subject breathed freely throughout the experiment. The breath-by-breath pulmonary gas exchange was measured and heart rate was estimated using an electrocardiogram. At the time of starting baseline exercise, $PetCO_2$ in PE and RE were lower than CE. During baseline exercise, $PetCO_2$ in RE increased, and $PetCO_2$ in PE was lower than RE and CE at the time of starting heavy exercise. Respiratory quotient in PE was higher than RE and CE during baseline exercise, and then decreased to lower than that in RE and CE during heavy exercise. It is possible that these differences between PE and RE have an effect on oxygen uptake kinetics.

(COI: No)

P2-248

Class III/IV POU transcription factors expressed in small cell lung cancer cells are involved in proneural/neuroendocrine differentiation

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One-third of lung malignancies demonstrate a proneural/neuroendocrine phenotype or type of differentiation. However, it has not been clearly elucidated how proneural/neuroendocrine differentiation is controlled in lung cancers. We recently demonstrated that the POU3F2 gene plays a significant role in proneural/neuroendocrine differentiation of lung cancers. Because class III POU genes (POU3F1, POU3F2, POU3F3, and POU3F4) and class IV POU genes (POU4F1, POU4F2, and POU4F3) share similar properties in neural development, we analyzed the association between class III/IV POU genes and a proneural/neuroendocrine phenotype in lung cancers using seven small cell lung cancer (SCLC) cell lines and twelve non-SCLC (NSCLC) cell lines. Class III/IV POU gene expression was generally restricted to SCLC cells. However, the forced expression of class III/IV POU genes in the NSCLC cell lines induced the expression of neuroendocrine-specific markers (neural call adhesion molecule 1, synaptophysin, and chromogranin A) and proneural transcription factors (achaete-scute homolog-like 1, NeuroD1, and thyroid transcription factor 1) in various degrees. Furthermore, each class III/IV POU gene induced other class III/IV POU genes, suggesting the mutual induction of class III/IV POU genes. These findings suggest that the expression of class III/IV POU genes is important for the proneural/neuroendocrine differentiation of lung cancer cells.

(COI: No)

P2-249

Sustained vocalizations suppress expiration of carbon dioxide during *kendo* exercises

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One of the distinct traits of *kendo*, the traditional Japanese martial art of fencing, is the execution of sustained, high-effort vocalizations during actions. The purpose of this study was to determine the effect of these vocalizations on respiratory functions. Respiratory indicators of eight university *kendo* athletes were analyzed using a portable breath gas analyzer during the most intensive *kendo* exercise, *kakari-keiko*, with and without vocalization. Breathing frequency (f_B) increased regardless of vocalization, but in trials with vocalization, f_B and expired minute ventilation were significantly smaller, and expiration time was significantly longer. Components of expired gases were also affected by vocalization: Although there was no significant difference in oxygen uptake, vocalization yielded a reduction in carbon dioxide output (VCO_2) and an increase in fraction of end-tidal carbon dioxide ($EtCO_2$). Thus, we conclude that these vocalizations greatly affect expiration breathing patterns in *kendo*. Moreover, repetition of *kakari-keiko* caused a reduction in VCO_2 and an increase in $EtCO_2$. We consider the possibility that the sustained high-effort vocalizations of *kendo* also increase cerebral blood flow.

(COI: No)

P2-250

Nasal but not tracheal TRPA1 contributes to irritant-induced respiratory slowing

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Transient receptor potential ankyrin 1 (TRPA1), a member of the TRP superfamily, exists in the sensory neurons including trigeminal neurons innervating nasal cavity (Sci Rep 3:3100, 2013) and vagal neurons innervating trachea (Nat Chem Biol 7:701, 2011). Although TRPA1 has been proposed as an irritant receptor of which stimulation triggers respiratory slowing, precise locations of responsible receptor have not been known. Here we examined relative importance of TRPA1 located in upper airway (nasal) and lower airway (trachea). Urethane (1.3-1.5 g/kg)-anesthetized male mice were studied. The trachea was sectioned just caudal to the thyroid cartilage. A cannula was inserted into the rostral sectioned trachea with its tip just passing through the posterior nasal aperture to the nasal cavity for upper airway stimulation. Another cannula was inserted into the caudal sectioned trachea for both ventilation and lower airway stimulation. Flow velocity of spontaneous breathing was measured with a Lilly type flow meter attached to one end of a T-tube inserted into the caudal sectioned trachea. A vapor of one of the TRPA1-stimulants, allyl isothiocyanate (AITC), was introduced into the airline by placing a cotton paper soaked with 20 uL of AITC solution (98%). AITC slowed the respiratory frequency when applied to the upper (ca -30%) but not to the lower airway (ca -5%). No response was observed in TRPA1 knockout mice. These data clearly show that TRPA1 in the nasal cavity is more important than that in the trachea for irritant-induced respiratory slowing.

(COI: No)

P2-251

Blockade of astrocytic activation augments hypoxia-induced depression of ventilation and EEG

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Although mild hypoxia increases ventilation, severe hypoxia disturbs consciousness and decreases ventilation, which makes the subject more hypoxic and eventually causes death. Respiratory motor output is mainly controlled in the brainstem. However, ventilatory response to hypoxia is also dependent on the higher brain status. Recent advances in glial physiology have demonstrated that astrocytes play important roles in information processing in various brain regions. Here we hypothesized that the maintenance of both the higher brain and the brainstem functions are critically dependent on the activity of astrocytes, and analyzed the effects of astrocytic activation blockade on the higher brain function and ventilation. In unanesthetized adult mice the higher brain status and ventilation were monitored by EEG and whole body plethysmography, respectively. Mild (12%) and severe (6%) hypoxia was loaded to mice before and after administration of arundic acid which blocked activation of astrocytes. Severe hypoxia-induced ventilatory depression was accompanied by disturbance of the higher brain that would decrease the central command to the brainstem respiratory center. Hypoxia-induced inhibition of EEG and ventilation was augmented with arundic acid. We suggest that astrocytes importantly contribute to the maintenance of the higher brain function and ventilation under hypoxia.

(COI: Properly Declared)

P2-252

Functional expression of TRPV4 receptor in mouse nasal epithelium

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The nasal epithelium consists of the olfactory and respiratory ciliated epithelia, both of which have sensory properties that are able to respond to various stimuli, such as odorants, carbon dioxide and physical pressures. Transient receptor potential vanilloid type 4 (TRPV4) is known to be a Ca^{2+}-permeable cation channels activated by various physical and chemical stimuli. However, TRPV4 expression in the nasal epithelium has not been previously investigated. We performed RT-PCR, *in situ* hybridization, immunohistochemistry and calcium imaging analysis using wild-type (WT) and TRPV4-knockout (TRPV4-KO) mice to examine the functional expression of TRPV4 channels in the nasal epithelium. TRPV4 mRNA was expressed in the nasal epithelial tissues. TRPV4-positive immunoreactions were observed in the basal cells in both the olfactory and ciliated epithelia. Calcium imaging analysis showed that 4 α-PDD, a TRPV4 agonist, increased an intracellular calcium concentration in a subset of dissociated nasal epithelial cells, indicating the presence of functional TRPV4 channels in the nasal epithelium. Taken altogether, functional TRPV4 is specifically expressed in the basal cells in both the olfactory and respiratory ciliated epithelia. Since TRPV4 is present in the basal cells of some stratified epithelia, such as the urothelium and esophageal epithelium, TRPV4 receptor may be a universal regulator of Ca^{2+}-dependent signaling pathways linked to cell proliferation, cell survival, ATP release, and cytokine production in the basal cells.

(COI: No)

P2-253

Loose regularities of calcium bursting sequence among inspiratory cells in the pre-Botzinger complex during rhythmic burst

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Spontaneous respiratory rhythm is essential for our life activity. Medullary slices containing the pre-Botzinger complex (preBotC) can preserve the spontaneous respiratory rhythmic activity. In the preBotC, respiratory cells including pacemaker cells activate stochastically with each rhythmic burst. In spite of the importance to understand the neuronal network structure and function, the manner of bursting sequence among respiratory cells has not been studied well. Here, we investigated bursting sequences among inspiratory cells in the preBotC during rhythmic bursts using wide-field calcium imaging. For the evaluation of rhythmic calcium bursting sequence, we adopted onset timing and peak timing of calcium fluctuation during rhythmic burst in individual cells. These two timings had weak correlation, which suggests that the parameters might reflect different physiological events. The sequences of both timing changed flexibly at each individual rhythmic burst, and the two kinds of sequences were also different from each other even in the same sequence. In addition, a subset of inspiratory cells covered the earliest activations in the sequences of both timings. These results suggest that the sequence of rhythmic calcium bursts has some loose regularities but is not invariable.

(COI: No)

P2-254

Light and Electron Microscopical Study on the Particulate Bodies Found in Epithelium of Rat Airway

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We found particulates in the epithelium of rat larynx and trachea, but the nature has never been clarified. In this study, we aimed to clarify the characteristics of these particulate.
MATERIALS and METHODS: Larynges, tracheae and lungs from 5 male Wistar rats were observed under a light microscope, and tracheae from 3 male Wistar rats were observed under transmission electron microscope.
RESULTS: The particulate in the epithelium of larynx and trachea were strongly positive for periodic acid Schiff reaction (PAS) and poorly stained with hematoxylin-eosin staining. Particulates with similar stainabilities were also found in the food. The particulates were surrounded by a flattened epithelial cell and frequently found in the ventral epithelium of larynx and in the dorsal epithelium of trachea. The epithelial cells which surrounded the particulates were non-ciliated cells. The microvilli were found in the surface face to the particulate. The secretory granules showing similar electron density with the particulates were found in the cytoplasm of the epithelial cells. The fusion of the particulate with the secretory substances secreted from epithelial cells was rarely found.
DISCUSSION: From the present findings, the origin of particulate bodies is discussed: the exogenous food-derived particulates inhaled into the airway or the intrinsic substances secreted from epithelial cells.

(COI: No)

P2-255

Simultaneous imaging of hemodynamics and hypoxia signals in lung tissues of mouse melanoma metastasis with in vivo cryotechnique

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Cancer cells in metastatic foci need blood supply to proliferate and develop, as those in their primary nest. In this study, we have clarified dynamic structures of blood vessels and circulation in lung metastatic tumor masses (mouse melanoma B16-BL6 cells). The relationship between blood supply and appearance of ischemia signal, HIF-1 α, was compared in primary tumor nests and metastatic tumor masses with in vivo cryotechnique (IVCT), which can capture animal cells and tissues without anoxia. The lung metastatic tissues could be categorized into three areas: (1) tumor masses with poor blood vessels, (2) tumor cells around large blood vessels, and (3) tumor tissues with abundant blood capillaries. In the area (1), melanoma cells were often localized near pleura, and their HIF-1 α was expressed equally in each nucleus, similar to the primary melanoma cells. In the area (3), blood capillaries were accompanied with elastic or collagen fibers, endothelia and type I epithelial cells, resembling the alveolar septum. Horseradish peroxidase injection also revealed that blood circulation was well maintained in these areas. Immunoreactivities of HIF-1 α were varied in the tumor masses, relating to surrounding type I epithelial cells. Therefore, oxygen of some lung metastatic tumors is supplied by original alveolar capillary structures, different from that in their primary nests.

(COI: No)

P2-256

The pulmonary artery remodeling during recovery phase in the hyperoxia-induced newborn lung injury ~Angiopoietin-1 might be therapeutic strategy in the injured developing lung~

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Bronchopulmoany dysplasia (BPD) is a chronic lung disease of premature infants associated with pulmonary alveolar and microvascular injury, and causes the long-term respiratory cardiac problems. In the previous study, we demonstrated that injured developing lung continues to have an abnormal alveolar and microvascular structure even after recovery period, and that Angiopoietin-1(Ang-1) might improve those structural changes. But we don't have enough information about pulmonary artery remodeling (PAR) in those models. And therefore we hypothesized that injured developing lung is associated with continuous abnormal PAR and Ang-1 treatment improves those changes, we investigated PAR with the parameter of medial thickness (MT), adventitial thickness (AT), and total wall thickness (WT) for stereological analysis, by using a hyperoxia-exposed mouse model of BPD. The recovery models still had increased distal air-space area, decreased abundance of secondary septae and thick blood-air barriers. MT%, AT%, and WT%, which increased in the hyperoxic lung, still continued to be larger in recovery models. On the other hand, Ang-1 treated lung partially improved those changes. Those results suggest that injured developing lung continues to have abnormal structure even in recovery period, which is correlated with high morbidity and mortality of BPD patients. Ang-1 might be some therapeutic approach in BPD.

(COI: No)

P2-257

Primary cilia localization of Nphp3 is responsible for renal function and morphology

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Nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease. More than a dozen genes have been identified that cause NPHP. The gene products of NPHP, nephrocystins, are presumed to function in the same pathway. Most of their products localize to cilium or centriole. Nephrocystins-1, -4, -5, -6 are localized to the transition zone of cilium or centriole, and proposed to function as ciliary barrier. Nephrocystin-2/inv, nephrocystin-3 and nephrocystin-9/nek8 are localized the proximal segment of the ciliary shaft named "Inv compartment". We previously reported that Nephrocystin-2/inv acts as an anchor for nephrocystin-3 and nek8. Therefore, it is likely that inv, nephrocystin-3 and nek8 make a functional complex. Although nephrocystins are localized in the cilia or centriole, it is still unknown if the localization of cilia or centriole is essential for their functions. N-terminal second glycine is also essential for trafficking of NPHP3 into the cilia. Replacement of the second glycine to alanine abolished ciliary translocation of nephrocystin-3. To understand the function of nephrocystin-3 in the cilia, we generated Nphp3 Gly2Ala (G2A) knock-in mouse. Two ES cell lines line 35 and 88, carrying the mutation, were generated and chimeric mice were made. The chimeric mice were mated with CAG-cre mice to remove the loxP-flanked neomycin selection cassette. We are going to present the phenotypes of the knock-in mouse mutants.

(COI: No)

P2-258

Involvement of podocin on abnormal receptor-response and mechanosensitivity of mutant TRPC6 channels associated with familial focal segmental glomerulosclerosis (FSGS)

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The mutations of transient receptor potential cation channel 6 (TRPC6) are causative to some hereditary forms of focal segmental glomeruloscleosis (FSGS). We previously investigated the functional impacts of murine TRPC6 FSGS mutations near its N-terminal ankyrin repeats (G108S, P111Q, N124S, M131T, N142S, R174Q) in heterologous expression system with Ca^{2+} imaging and patch clamp techniques, and found that some of these mutants showed enhanced receptor responses to carbachol and different mechanosensitivity. In this study, we investigated the role of podocin, a slit diaphragm protein and a putative mechanosensor, on the receptor responses and mechanosensitivity of TRPC6 FSGS mutants. Co-expression of podocin with TRPC6 in HEK293 cells enhanced responses to low-concentration of carbachol (1 μM) in wild-type, P111Q and M131T, but not in N142S. Mechanical responses cause by a membrane-expanding agent 2, 4, 6-trinitrophenol in these mutants were differently affected by podocin. We also studied the effects of overexpression of TRPC6 FSGS mutants in mouse-derived podocyte cell line which is abundant with podocin. M131T-overexpressed podocytes showed higher basal Ca^{2+} levels than wild-type. These observations indicate that podocin differently modulates the receptor activation and mechanosensitivity of TRPC6 FSGS mutants. Such differences may reflect the different etiology of FSGS patients such as the onset age of diseases, where disruption of filtration barrier by the degeneration of podocytes play a crucial role.

(COI: No)

P2-259

Dynamic fluctuation of glomerular fenestrated endocapillary

Chiba, Seiichi[1]; Arai, Akihito[1,2]; Aosa, Taishi[1,3]; Tatsukawa, Shuji[1]; Ina, Keisuke[1]; Fujikura, Yoshihisa[1] (*[1]Oita Univ., Oita, Japan; [2]Oita Univ., Oita, Japan; [3]Oita Univ., Oita, Japan*)

Fenestrated endocapillary regulates transduction of nutrients and humoral information between the systemic and peripheral cells via micropore. In this study, we focused on the morphological changes of the micropores of glomerular fenestrated endocapillaries according to relative hypoglycemic stress induced by chronic continuous subcutaneous insulin injection. Male mice were divided into three group; free-feeding and saline-injected group as a control (CS), free-feeding and insulin-injected group (CI), and scheduled-feeding and insulin-injected group (SI), respectively. The changes in body weight, daily amount of feeding, urinal protein exclusion, and rectal temperature, blood glucose variation and blood pressure were also obtained. Subsequently, all mice were perfused and removed the kidney samples for the microscopic investigation. All statistical significance was set at $p < 0.05$. As a result, significant elevations were observed in CS compared to CI of the parameters of body weight, daily feeding and urinal protein exclusion, blood glucose variation, rectal temperature and blood pressure. On the other hand, the elevation of the rectal temperature and blood pressure and the reduction of the blood glucose variation were confirmed significantly in SI compared to CI, respectively. In the electron microscopic imaging analysis, we ascertained the significant increment in the mean diameter of the micropores in CI and SI compared to CS. SI had outclassed CI in the magnitude of diameter of the micropore significantly.

(COI: No)

P2-260

Newly characterized structure of podocytes revealed by three-dimensional analysis using block-face scanning electron microscopy

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Block-face imaging is a novel SEM technique which enables easier acquisition of serial ultrastructural images directly from the surface of resin-embedded biological samples with a similar quality to TEM images. In the present study, we analyzed the three-dimensional architecture of podocytes using serial block-face imaging. When the reconstructed podocytes from their basal side were viewed, the most proximal portion of the foot processes were connected to each other via a tortuous ridge-like prominence, which was formed on the undersurface of the primary process and was similar to the usual foot processes in structure. We termed the ridge-like prominence a "connecting foot process", and to distinguish the connecting foot processes from the usual ones, we further named the latter as "peripheral foot processes". The connecting foot process anchored the primary process to the glomerular basement membrane, and connected the primary process and the peripheral foot processes. In conclusion, serial block-face imaging is a powerful tool for understanding the three-dimensional architecture of podocytes through its ability to reveal novel structures which were difficult to determine by conventional TEM and SEM alone.

(COI: No)

P2-261

Segment-specific expression of tight junctional proteins, claudins, is regulated by osmotic stress in renal tubular epithelial cells

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Tight junctions form the closest contact between adjacent cells. Claudin-2, a transmembrane protein of tight junction, is expressed in the proximal tubules where maintain an isotonic osmolarity, whereas claudin-4 is expressed in the collecting ducts where maintain a high osmolarity in the fluid of the kidney. In this study, we examined the effect of hyperosmolarity on claudins expression. Hyperosmolarity increased claudin-4 expression, whereas it decreased claudin-2 expression in MDCK II cells. Claudin-4 expression is up-regulated by a MEK/ERK pathway under the hyperosmotic conditions, whereas claudin-2 expression is not down-regulated. We found that the hyperosmolarity-induced decrease in claudin-2 is inhibited by a PKCβ specific inhibitor in MDCK II cells, rat renal slices, and HK-2 human proximal tubular cells. Hyperosmolarity decreased the expression of nuclear GATA-2, which was inhibited by Go6983 and PKCβ inhibitor. Chromatin immunoprecipitation assay showed that GATA-2 bound to the promoter region of claudin-2. Hyperosmolarity may decrease claudin-2 expression mediated by a decrease in PKCβ-dependent GATA-2 transcriptional activity in renal tubular epithelial cells. We suggest that the decrease in claudin-2 expression prevents excess paracellular transport of electrolytes under the hyperosmotic conditions.

(COI: No)

P2-262

Three-dimensional morphological analysis of Alport syndrome and thin basement membrane nephropathy by low vacuum scanning electron microscopy

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We introduce a novel approach to the histological diagnosis of Alport syndrome (AS) and thin basement membrane nephropathy (TBMN) in the present study. These diseases are caused by mutations of the type IV collagen genes. We investigated the three-dimensional ultrastructure of the glomerular basement membrane (GBM) of AS and TBMN under low vacuum scanning electron microscopy (LVSEM). Conventional renal biopsy paraffin sections obtained from 4 cases of AS and 6 cases of TBMN patients were stained with periodic acid methenamine silver (PAM) and observed under LVSEM. The PAM-positive GBM was clearly visible under LVSEM through the overlying cellular components without removing of cells. The GBM showed characteristic coarse meshwork appearances in AS, and thin and sheet-like appearances in TBMN. Moreover, at the cut side view of the capillary wall, the GBM in AS appeared as fibrous inclusions between podocytes and endothelial cells. These different findings of GBM between AS and TBMN were considered to be depending on the different constitutions of alpha chains of collagen type IV. The present three-dimensional morphological analysis of GBM by LVSEM using conventional paraffin sections is probably useful to the histological diagnosis of AS and TBMN, including in retrospective investigations.

(COI: No)

P2-263

Search results for novel biomarker in acute kidney injury

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Acute kidney injury (AKI) is caused by various events, for example ischemia-reperfusion and drug administration, and despite the advances in renal replacement therapy, the mortality rate still remains high. Definitions of AKI have relied on either an abrupt increase in serum creatinine or an abrupt decline in urine output within 48h. Multiple studies have focused on the developement of the selective and specific biomarker in the early checkup of AKI. However, it was not reported in most biomarkers to predict a renal prognosis. The aim of the present study was searched novel biomarkers for prognosis prediction in AKI. Mice were subjected to 45 and 60 min of unilateral IRI. Kidney weight/body weight (KW/BW) in the 45-min IRI groups were the same as that of the sham group, whereas KW/BW in the 60-min IRI groups were significantly lower than that of the sham group at 28 days after IRI. From these results, we selected the kidneys that received 60 min of ischemia as the atrophic kidney model, and the kidneys that received 45 min of ischemia as the repaired kidney model. Next, we compared the two models in cDNA microarray method. Microarray experiments were performed using amplified RNA from two IRI mouse kidneys and sham control tissue at 1 day after operation. Number of the up-regulated gene is 177 in the atrophic model only. We have been made the gene expression profiles. It is expected that the novel biomarker is finding out.

(COI: No)

P2-264

Protective effect of renal denervation on renal ischemia-reperfusion injury in rats

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This study was carried out to investigate the involvement of renal innervation in renal injury and repair after ischemia-reperfusion (IR). IR-induced acute kidney injury was induced by unilateral clamping of the renal artery and vein for 30 min followed by reperfusion for 1, 2, 4, and 7 days, after the contralateral nephrectomy. Unilateral renal denervation was performed by cutting large visible nerves and applying phenol. IR significantly increased necrosis of tubular epithelial cells, interstitial expansion, collagen deposition, at the cortico-medullary border. IR also increased the formation of reactive oxygen species in the same region as detected by dihydroethidium staining. Renal denervation improved histological damage and decreased the formation of reactive oxygen species. These results suggest that renal nerves play an important role in the regulation of the progression of IR injury, and this regulation is associated with the attenuation of oxidative stress.

(COI: No)

P2-265

The localization of vacuolar H+-ATPases (v-ATPases) in proximal tubules

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We previously reported that increasing basolateral CO2 from 1.5 to 5% at the constant HCO3- concentration induced slight increases in the cytosolic pH (pHc), the luminal fluid in the proximal tubule (pHTF), and the fluorescence intensity of acridine orange in acid vesicles. Bafilomycin at 10-6 M inhibited these changes. To clarify the mechanism of pH regulation by the v-ATPase and the CLC-5 (H/Cl exchange transporter 5), we examined the reactivity of anti v-ATPase and CLC-5 antibodies in bullfrog proximal tubules. Localized signals were observed, both for the v-ATPase and the CLC-5, on the cytoplasmic membrane and cytosolic vesicles. In response to the 5% basolateral CO2, however, localized antibody signals did not change. Furthermore, mRNA and protein levels remained unchanged. These results suggested the activity of v-ATPase may be responsible for the v-ATPase-dependent pH regulation in proximal tubules.

(COI: No)

P2-266

Properties of expression of exogenously transfected ROMK K+ channels with or without PDZ-binding motif in the polarized and non-polarized membranes of cultured M-1 cell

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ROMK1 K+ channels are expressed in apical side of cortical collecting duct principal cells and play an essential role in K+ secretion to maintain body fluid potassium homeostasis. In this study, to understand the mechanism of specific expression of ROMK1 channel on apical membrane, we focused on PDZ-binding motif at C-terminus of ROMK1 channel. PDZ binding motif has been known to be important for interaction with PDZ scaffold proteins regulating ion channel expression on cell membrane. We constructed EGFP fused ROMK1 deleted PDZ binding motif and transfected to cultured M-1 cells. We applied two types of culture conditions. One was M-1 cells cultured on conventional glass dish, and the other was cells cultured on membrane insert to form apico-basolateral cell polarity. Cell attached mode of patch clamp analysis confirmed that ROMK1 activity was observed in EGFP positive M-1 cells, but not EGFP negative cells. No obvious difference between WT and PDZ binding motif deletion mutant in cellular localization of EGFP fluorescence and detection of frequency of ROMK1 current in polarized M-1 cell. On the other hand, frequency of ROMK1 current acquisition was significantly decreased in deletion mutant compared with WT in non-polarized cell. These results suggest that WT and deletion mutant, both of which have high affinity to polarized apical membrane.

(COI: No)

P2-267

Myogenic damage underlies dysfunctional urethral closure in a rat model of urinary incontinence

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Purpose: During pregnancy and/or after childbirth, many women suffer from stress urinary incontinence (SUI). Various animal models exist that have contributed to finding a suitable therapeutic method to investigate SUI pathophysiology. However, few anatomical studies have provided detailed information on the structures involved. The aim of this study was to examine the effect of simulated birth trauma on the urethral wall musculature including both striated and smooth muscle layers using the rat vaginal distention (VD) model of SUI.

Methods: Female, 8-week-old Wistar rats were divided into control and VD groups. In the VD group, a urinary catheter was placed into the vagina and the balloon was inflated for 4 hours to simulate labor. After 4 weeks, urethral tissue samples were collected for histochemical analysis. Immunofluorescence staining was conducted to detect striated and smooth muscle fibers in the urethral wall using antibodies for myosin heavy chain isoforms (type I, type II) and α-smooth muscle actin.

Results: In general, the most outer part of urethral wall contained a circular striated muscle layer predominantly consisting of type II fibers. Just beneath this layer, a thin circular smooth muscle layer enclosed the mucosa. The VD group showed a marked decrease in the urethral wall musculature of both skeletal and smooth muscle tissues compared to that in the control group.

Conclusion: The VD rat model is useful for examining myogenic damage associated with SUI in humans.

(COI: No)

P2-268

Pentraxin3 expression in a rat model of peritoneal dialysis

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Background: Continuous exposure to peritoneal dialysis (PD) fluid is associated with micro-inflammation that leads to tissue fibrosis of the peritoneum and failure of peritoneal membrane ultrafiltration. Pentraxin3 (PTX3) is a multifunctional soluble pattern recognition receptor modulating the immune-inflammatory responses. PTX3 is produced at sites of inflammation by various cell types. We investigated whether PTX3 could be a marker of inflammation for the peritoneum.

Methods: The rats were instilled with 20 ml of lactate-buffered PD fluid containing 3.86% glucose (n=7) or saline (n=6) twice a day for 8 weeks. The mRNA expression of PTX3 in the parietal peritoneum was evaluated by RT-PCR, real-time PCR and in situ hybridization, and was compared to the expression level in saline group and normal group without PD (n=6). We also examined PTX3 expression in several types of cultured cell line.

Results: Morphological data revealed that the submesothelial layer of peritoneal membranes in PD rats was markedly thickened with fibrosis and angiogenesis. The expression of PTX3 was detected and enhanced in the peritoneal tissues by continuous exposure to conventional PD fluid. PTX3 was also induced in cultured mesothelial cells as well as macrophage-like cells and fibroblasts.

Conclusion: PTX3 might be a potential biological marker of local micro-inflammation in the peritoneal tissue undergoing PD therapy.

(COI: No)

P2-269

Properties of spontaneous activity in muscularis mucosa of the guinea pig bladder

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Aim: Properties of spontaneous activity in urothelium denuded muscularis mucosa (MM) of the guinea-pig bladder were investigated.

Methods: Effects of ion channel modulators on spontaneous action potentials (SAPs) were investigated using intracellular recording technique, while spontaneous Ca2+ transients were visualized with fluo-4 Ca2+ imaging. Fluorescent immunohistochemistry was also carried out.

Results: The resting membrane potential of MM was -45.3 ± 5.5mV. MM generated SPAs that had the amplitude of 46.7 ± 4.5mV and the frequency of 12.3 ± 9.5/min. Both SAPs and spontaneous Ca2+ transients were abolished by nifedipine. NS 309, a small-conductance Ca2+ activated K+ channel opener, prolonged SAP after-hyperpolarizations and its action was reversed by apamin. NS 1619, a large conductance Ca2+ activated K+ channel opener, caused hyperpolarizations and cessation of SAPs in iberiotoxin-sensitive manner. Y-26763, an ATP-sensitive K+ channel opener, hyperpolarized the membrane and abolished SAPs, and its actions were reversed by glibenclamide. α-smooth muscle actin positive MM bundles forming mesh-like network preferentially run along suburothelial vessels.

Conclusions: SAPs and Ca2+ transients in MM rely on the opening of L-type Ca2+ channels, while the activation of K+ channels results in the suppression of SAPs, and thus properties of spontanepus activity in MM is very similar to that of detrusor smooth muscle. Spontaneous contractions of MM may prevent stretching the vessels in their long axis during urine storage phase.

(COI: No)

P2-270

Regulation of hyperactivated motility by extracellular Na^+ in hamster spermatozoa

Takei, Gen Leon; Fujinoki, Masakatsu; Seo, Yoshiteru (*Dokkyo Medical University Tochigi Japan*)

Mammalian spermatozoa have to undergo "capacitation" to become fertilization-competent. Capacitated spermatozoa exhibit a specialized flagellar movement called "hyperactivation" with increased bend amplitude to penetrate the zona pellucida. In mammals, osmolalities of the fluids from male internal genitalia (seminal vesicle, prostate and epididymis) are higher (<420 mOs*m*) than that of the fluids from female genital tract (approximately 290 mOs*m*). We previously reported that the appearance of hamster sperm hyperactivation was delayed near the osmolality of seminal plasma using mTALP media in which osmolality were adjusted by NaCl. In the present study, we examined whether the delay of hamster sperm hyperactivation was caused by the osmolality or the concentration extracellular Na^+ of the mTALP media. To examine this, spermatozoa were incubated in the mTALP media in which NaCl concentrations were varied from 75 mM to 150 mM, while the osmolalities were fixed at 370 mOs*m* by adding mannitol. The results indicated that the delay of hyperactivation was caused dependently on the concentration of extracellular Na^+, but not the osmolality of the mTALP media. Intracellular Ca^{2+} concentration was decreased as the extracellular Na^+ concentration increased. By contrast, the membrane potential and intracellular pH were not affected by the extracellular Na^+ concentration. SN-6, an inhibitor of Na^+/Ca^{2+} exchanger (NCX), canceled the delay of hyperactivation in the presence of suppressive concentration of Na^+. These data suggest that mammalian sperm hyperactivation is regulated by extracellular Na+ by the action of NCX.

(COI: No)

P2-271

Searching the enzyme responsible for molecular weight reduction of sperm acrosomal protein Equatorin during acrosome reaction

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Equatorin (EQTN) is a heavily glycosylated single transmembrane protein which is localized on the equatorial segment of sperm acrosome. Sperm-egg fusion occurs between the membrane over the equatorial segment and the oolemma only after acrosome reaction. Anti-EQTN antibody MN9 inhibits cortical granule release without inhibiting zona penetration during fertilization. This suggests that EQTN is involved in sperm-egg interaction and early phase of egg activation. Relative molecular weight of EQTN changes during sperm maturation and acrosome reaction. Therefore, it is important to reveal the post-translational modifications of EQTN to understand the molecular mechanism of sperm-egg interaction and egg activation. In this study, we examined the molecular weight shift of EQTN during acrosome reaction. Mild detergent treatments as well as acrosome reaction were able to induce relative molecular weight reduction of EQTN. To detect the enzyme activity responsible for the molecular weight shift, we extracted sperm proteins using mild detergents and mixed with EQTN as substrate. The molecular weight shifts were detected by western blotting. We purified the enzyme responsible for the reduction of EQTN molecular weight to analyze the function during fertilization. Identification of the enzyme will elucidate the function of the fragment cleaved from EQTN which could not be analyzed by EQTN knock out mice.

(COI: No)

P2-272

Basigin interacts with monocarboxylate transporter 2 in the mouse testes and sperm

Maekawa, Mamiko; Chen, Cheng; Yamatoya, Kenji; Ito, Chizuru; Toshimori, Kiyotaka (*Grad. Sch. Med. Chiba Uniiv., Chiba, Japan*)

Basigin (CD147, EMMPRIN) is a member of the immunoglobulin superfamily and plays various important roles in biological events including spermatogenesis. It is known that basigin is associated with monocarboxylate transporters (MCTs), especially with MCT1 in some cell types. However, the interaction of basigin with MCTs in the testis and sperm has not yet been clarified. Using antibodies against MCT1, MCT2 and basigin, we investigated the localization of the MCTs and basigin in the mouse testis and sperm. During testicular development MCT1 immunoreactivity was localized on the spermatogonia, spermatocytes, and spermatids. Immunostaining for MCT2 was detected in elongate spermatids and the principal piece of the sperm tails in the testis. Basigin immunoreactivity was observed on the spermatocytes, spermatids and principal piece of the testicular sperm. An indirect immunofluorescence study revealed protein localization in sperm from caput and cauda epididymides. Immunoreactivities for basigin and MCT2 were colocalized; in sperm from the caput epididymidis, the reactions occurred on the principal piece of the sperm tail. In contrast, the midpiece of the sperm tail was positive for basigin and MCT2 in sperm from the cauda epididymidis. Furthermore, MCT2 was immunoprecipitated with basigin in mouse testes and sperm. These results indicate that basigin preferably binds with MCT2, not with MCT1, in testicular and epididymal sperm.

(COI: No)

P2-273

Establishment of in vitro differentiation system of male germ stem cells by epigenetic manipulation

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Male germ lineage in mice has an exceptional adult type stem cell system that the stem cells are maintained for years in vitro. Although the long-term cultured germ stem cells, called GS cells, are able to reconstitute all of the testicular germ cells in recipient adult testes, in vitro differentiation system has not been well established. In order to dissect out the molecular mechanisms how stem cells are maintained and differentiate, the establishment of in vitro differentiation system is desired. We previously reported that the stem cells in testes lacked the expression of de novo DNA methyltransferases (Dnmts) and revealed weak global H3K9me2 modification. The protein expression of Dnmts and integration of H3K9me2 modification were induced at the transition from the stem cells to the progenitor cells. We are currently trying to establish in vitro differentiation system by regulating the epigenetic modifications and mimicking the epigenetic status in vivo. We will discuss about whether it is possible to regulate stem cell differentiation by low molecular weight compounds that regulate epigenetic modification enzymes.

(COI: No)

P2-274

Ca^{2+} oscillations of mouse ovarian oocytes can be used as a cytotoxicity assay system for drugs and chemicals

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Background: Cytotoxicity assays for chemicals and drugs are usually carried out by monitoring cell viability or occasionally by checking morphologic or biochemical changes induced in cells. Here we present an assay system which continuously monitors alterations in cell function in response to these substances.
Methods: Ovarian oocytes were obtained from mature ICR mice, loaded with Fura-2/AM and their fluorescence Ca^{2+} images were monitored using an image analyser (ARGUS-50, Hamamatsu Photonics, Japan).
Results: 1) Among medicines, lidocaine (local anesthetic, antiarrhythmic) and phenytoin (antiepileptic) were tested. At therapeutic concentrations, these drugs did not reveal any significant effects on Ca^{2+} oscillations. However, oscillations were reversibly blocked by lidocaine and irreversibly by phenytoin when their concentration exceeded the therapeutic range. 2) Cadmium, an occupational and environmental pollutant, also inhibited Ca^{2+} oscillations reversibly at low concentrations (<0.01 mM) and irreversibly at higher doses (>0.1 mM). 3) Uncouplers of oxidative phosphorylation in mitochondria, e.g. CCCP (100 nM), reversibly inhibited Ca^{2+} oscillations.
Conclusion: The present work shows that Ca^{2+} dynamics of mouse oocytes provides a cytotoxicity assay system for drug screening. This system can also be applied to detect hazardous substances which may cause occupational or environmental diseases.

(COI: No)

P2-275

Kinetics of oocyte elimination by synapsis checkpoint in mice

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Synapsis checkpoint is an essential mechanism to ensure the quality of oocytes. We recently revealed that synapsis checkpoint requires HORMAD2 in mice. However, its mechanism is still poorly understood. The timing of the oocyte elimination is important for the analysis of synapsis checkpoint-associated proteins, but has not been well determined. In the present study, we first examined the kinetics of oocyte cell death during the perinatal period in wild-type, HORMAD1-hetero deficient (having oocytes with mild asynapsis), and SPO11-deficient (having oocytes with extensive asynapsis) ovaries by using cleaved PARP-1 as a marker of apoptosis. The percentage of apoptotic oocytes was similar among all the ovaries examined at 18 dpc (about 2 %), and slightly increased at 0 and 1 dpp in HORMAD1-hetero deficient (about 5 %) and SPO11-deficient (about 5 %) ovaries compared to wild-type (about 2 %) ovaries. However, the detected increase of apoptosis seemed to be relatively small compared to the final rate of oocyte loss in HORMAD1-hetero deficient (about 50 %) and SPO11-deficient (about 90 %) ovaries. We speculate from these data that the oocyte elimination might be dependent on both apoptotic and non-apoptotic mechanisms. Next, we preliminarily examined the possible involvement of autophagic cell death by using LC3 as a marker of the autophagy activation, but detected no increase in the LC3 accumulation in SPO11-deficient oocytes. Although the analysis is still in progress, our analysis will provide fundamental data necessary for the molecular characterization of synapsis checkpoint in mammalian oocytes.

(COI: No)

P2-276

Distribution of prosaposin and its receptors in rat uterus

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Prosaposin (PS) is a trophic factor and activator of sphingolipid hydrolase in lysosomes. G protein-coupled receptor (GPR) 37 and GPR37L1 are receptors for prosaptide and prosaposin. We generated a specific antibody to PS and examined the spatiotemporal distribution of both PS-immunoreactive (PS-IR) cells and prosaposin receptors in Wistar rat uterus. Immunoblotting using uterine tissue showed that the production of PS and its receptors was affected by the estrus cycle. PS-IR cells were distributed in the functional layer of the endometrium. Uterine epithelial and glandular tissues reacted strongly with the anti-PS antibody. To identify PS-IR cells, double and triple immunostaining were performed with antibodies against PS, CD68, and OX62. Large numbers of double- and triple-positive cells were detected, suggesting that antigen-presenting cells in the uterus contain abundant PS. GPR37- and GPR37L1-immunoreactive cells were distributed in the functional layer of the endometrium. Intense expression of PS mRNA, examined using *in situ* hybridization, was observed in rat uterus. In rats, alternative splicing generates two forms of mRNA coding for PS: Pro+9, containing a nine-base insertion, and Pro+0, which lacks the insertion. We examined the expression patterns of both forms of PS mRNA in rat uterus. Both types of mRNA (Pro+9 and Pro+0) were detected, indicating that rat uterus contains various types of PS-producing and/or -secreting cells. These findings suggest diverse pivotal functions for PS in the reproductive system.

(COI: No)

P2-277

Lgr4 is required for endometrial receptivity acquired through progesterone signaling

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LGR4 (leucine-rich repeat-containing G protein-coupled receptor 4) is identified novel family member of glycoprotein type GPCR (G protein coupled receptor) that have LHR and FSHR. We generated *Lgr4* conditional knocked-out mouse ($Lgr4^{K5\ KO}$) using the *Keratin5-Cre* mouse model. These female mice was shown that *Lgr4* exhibited the subfertility with defect of *Lgr4* in the uterine luminal epithelial. There, we focused endometrial receptivity which is the most important event during implantation because the ovarian function is normal in the $Lgr4^{K5\ KO}$ mice. In the early pregnancy hormonal state, the ovarian steroid hormone, progesterone (P4) and estrogen (E2), regulate transition of luminal epithelium to allow blastocysts implantation. Then, to the normal ($Lgr4^{K5\ Ctrl}$) and $Lgr4^{K5\ KO}$ mice, we observed how luminal epithelium would react when both mice was induced receptive stage by administration E2 and P4. Compared with $Lgr4^{K5\ Ctrl}$ mice, proliferative luminal cells were remained in $Lgr4^{K5\ KO}$ undergo P4 stimulation. In addition, the amount of phospho-progesterone receptor in $Lgr4^{K5\ KO}$ was lower and also progesterone receptor target genes, *ihh* and *areg*, was reduced. Together, our result suggested that LGR4 is contributed endometrial receptivity through the P4 signaling and we are continuing further analysis.

(COI: No)

P2-278

Plastic changes of sympathetic vasoconstrictor activity in the ovary by long-term treatment of estrogen

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It has been reported that the reduction in uterine sympathetic innervation is observed, when the serum estrogen level is high during the estrous cycle and pregnancy. In the ovary, the sympathetic innervation may also be suppressed by estrogen to keep good environment for the follicle development. The present study examined the effects of long-term treatment of estrogen on sympathetic vasoconstrictor activity in the ovary. Non pregnant Wister rats received sustained subcutaneous estrogen (water soluble 17-beta estradiol, 5 μg/kg/day) or saline for 2 or 4 weeks. The rats were anesthetized, and artificially ventilated. Respiration, body temperature, and mean arterial blood pressure were maintained at physiological level. The ovarian blood flow was measured using a laser Doppler flowmeter. The ovarian sympathetic nerve (the superior ovarian nerve: SON) was electrically stimulated at the supra-maximal intensity for C-fibers. Electrical stimulation of the SON produced stimulus frequency (1-50 Hz) dependent decrease in the ovarian blood flow in both saline-treated and estrogen-treated rats. However, the attenuated response curves was observed in estradiol-treated rats. This attenuation was more evident especially at low frequency stimulation (2-5 Hz), and also in 4-week treated rats than 2-week treated rats. The results revealed that long-term treatment of estrogen caused plastic changes in sympathetic vasoconstrictor activity in the ovary.

(COI: No)

P2-279

The role of cytokines in the interaction between sperm and cumulus cells

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Eutherian eggs are surrounded by a thick cumulus cell layer. Although cumulus cell-derived factors have been shown to promote fertilization, the molecular mechanism of the promoting effect is unclear. We have shown previously that mouse sperm acrosome contains pituitary adenylate cyclase-activating polypeptide (PACAP) which reacts to PAC1, a PACAP specific receptor, on the plasma membrane of cumulus cells, and stimulates the cells to secrete fertilization-promoting factors. Here, we performed gene expression profiling on cumulus to identify these factors. A total of 166 genes were differentially expressed after 1-h incubation with PACAP; 162 out of 166 genes were upregulated. Among them, we focused on the genes encoding cytokines, including chemokines (CCL2, CCL3, CXCL1, CXCL7), growth factors (HB-EGF, FGF1), and neuropeptides (neurokinin A, substance P). Immunofluorescence analysis showed that CCR1, a chemokine receptor for CCL3, and NK2, a neurokinin A receptor, were both localized on the anterior acrosome and midpiece of flagellum. This result suggests that these cytokines mediate crosstalk between sperm and cumulus cells, and are involved in the processes in fertilization.

(COI: No)

P2-280

ERα Signal Transduction Pathways Controlling Cell Size

Li, Zhonglian; Otsuki, Yoshinori (*Osaka Med. Col. Takatsuki, Osaka, Japan*)

Together with estradiol in human endometrium, estrogen receptor alpha (ER α) plays a critical role through its target molecules in control of proliferation, differentiation, invasion, and migration. Current data support the idea that estrogen receptor alpha (ER α located in cell membrane, cytoplasm, and nucleus collaboratively plays its important roles. The idea suggests that the key functions of ER α might vary in its subcellular locations. To avoid the effects of the subcellular translocations of ER α during analysis, we have allocated ER α to different subcellular locations in ER α-negative Ishikawa cell, using permanent transfection technique. The results from flow cytometry showed that the ER α-negative cells became significantly larger in size following genomic insertion of ER α. While the cytoplasmic ER α has the least impact, the largest cells in size were observed in these carrying the nuclear ER α. Further analysis revealed that the amount of expression was increased in such molecules as pS235/236-S6 Ribosomal Protein and pS2448-mTOR. We also demonstrated that cells with the nuclear ER α showed the best balance between the cell size and its proliferation. Our study indicates that through mTOR pathway, ER α play an important role in regulation of the endometrial cell size.

(COI: No)

P2-281

Sex-related genes' asymmetric expression in early mouse gonads

Umemura, Yuria[1]; Hashimoto, Rie[1]; Omotehara, Takuya[1]; Hirano, Tetsushi[1]; Mantani, Youhei[2]; Yokoyama, Toshifumi[1]; Kitagawa, Hiroshi[2]; Hoshi, Nobuhiko[1] (*[1]Lab. Mol. Morphol., Grad. Sch. Agr. Sci., Kobe Univ., Kobe, Japan; [2] Lab. Histophysiol., Grad. Sch. Agr. Sci., Kobe Univ., Kobe, Japan*)

Most male mammals have a symmetrical pair of testes and females a symmetrical pair of ovaries. Our transgenic mice (C57BL/6-Ypos) showing sex reversal produced true hermaphrodites, many of which had a testis on the left and an ovary on the right. We suspect that these true hermaphrodites are the result of the asymmetric expression of sex-related genes during a critical window of sex determination, and here we investigated the spatiotemporal changes in the expression of genes *Sry*, *Sox9*, *Fgf9* and *Wnt4* in the XY gonads at embryonic day 11.5 by qRT-PCR and whole-mount immunohistochemistry (Wmt-IHC). qRT-PCR showed that the peak *Sry* expression and increased expression of *Sox9* were earlier in the left gonad than the right. No significant difference was found between the right and left gonads in *Fgf9* and *Wnt4* expression. Wmt-IHC showed that the left gonad's Sox9-positive cells were distributed earlier than the right, at the end of the caudal region. Such minor asymmetric differences do not seem to have much influence on normal testis differentiation. In C57BL/6 mice, whose background is sensitive to genetic disruption, the knock-out of specific genes reportedly leads to XY gonadal sex reversal with true hermaphrodites. These results suggest that the asymmetric differences of sex-related gene expressions contribute to the true hermaphrodites unless some genes are expressed normally.

(COI: No)

P2-282

Selection of stable reference genes for quantitative RT-PCR analyses in developing mouse gonads

Yokoyama, Toshifumi[1]; Hashimoto, Rie[1]; Umemura, Yuria[1]; Omotehara, Takuya[1]; Nagahara, Daichi[1]; Hirano, Tetsushi[1]; Yanai, Shogo[1]; Minami, Kiichi[1]; Kubota, Naoto[1]; Mantani, Youhei[2]; Kitagawa, Hiroshi[2]; Hoshi, Nobuhiko[1] (*[1]Lab. Mol. Morphol., Grad. Sch. Agr. Sci., Kobe Univ., Kobe, Japan; [2]Lab. Histophysiol., Grad. Sch. Agr. Sci., Kobe Univ., Kobe, Japan*)

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) is the most widely used method for studying quantitative gene expression. An important factor when using the qRT-PCR method is the stability of the expression of reference genes; this expression should be stable regardless of changes in the developing stage or the sex. However, it is difficult to know the appropriate normalization gene. Here we report the selection of reference genes for the accurate normalization of quantitative gene expression data in developing mouse gonads from 10.5 days post coitum (dpc) to the adult stage (10.5, 11.5, 12.5 13.5, 15.5, 17.5 dpc, neonatal, 5 and 34 wks). We evaluated the expression stabilities of the genes *Gapdh*, *Hprt1*, *Actb*, *B2m*, *Tbp*, *Ppia*, *Tfrc*, *Gusb*, *Pgk1*, *Ubc*, *Ywhaz*, *Polr2a*, *Rplp0*, *18S* and *Sdha*. To identify the best candidate genes, we combined the scores of three software programs: Bestkeeper, Normfinder, and Genorm. The results suggest *Ppia*, *Polr2a* and *Pplp0* as reference genes to be used in experiments with fetal to adult mouse gonads. In contrast, we identified *Hprt1*, *Tbp* and *Tfrc* as reference genes to be used in experiments with fetal mouse gonads.

(COI: No)

P2-283

Effect of taxol on chromosomal cohesion and blastocyst formation rate in human oocytes

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OBJECTIVE: Premature chromatid separation (PCS), a common condition that results in pre-implantation embryo loss, becomes more prevalent in human oocytes with maternal age. Therefore we attempted to suppress PCS artificially in order to improve post-fertilization development.
METHODS: Chromosome aberrations, oocyte development, and tubulin-associating protein expression were examined in human oocytes that had been produced by in vitro maturation (IVM) either with or without taxol. GV oocytes were exposed to taxol for either 1 or 2 hr and cultured overnight in order to allow them to mature. Chromosome analysis was performed in oocytes treated with 5-100ng/ml taxol for 1 or 2 hr. For blastocyst rates, taxol-treated oocytes were inseminated with the partner's sperm. Immunofluorescent microscopy was used in at least 5 oocytes to detect tubulin and aurora proteins.
RESULTS: Taxol-treated oocytes were associated with a significant reduction in PCS frequency and a significant increase in blastocyst rate. The cortical tubulin network was thicker in taxol-treated GV oocytes than control GV oocytes. Phospho-aurora expression appeared to be up-regulated in the germinal vesicle karyoplasm and chromatin region soon after taxol treatment.
CONCLUSION: Taxol treatment at the germinal vesicle (GV) stage suppressed PCS, resulting in an increased blastocyst formation rate in in-vitro mature oocytes. The result also indicated that tubulin polymerization during prophase may contribute to phospho-aurora kinase localization during meiosis in human oocytes.

(COI: No)

P2-284

Aromatherapy improves sleep quality during the menstrual cycle of healthy women

Fujita, Sayaka (*The University of Shimane*)

The present study aimed to clarify the effects of aromatherapy on sleep quality during the menstrual cycle of healthy women. Sleep quality during the menstrual cycle of 29 women (non-aromatherapy group, n=18; aromatherapy group, n=11) was measured for about 1 month. Basal body temperature during the ovarian follicular, corpus luteum and menses phases was measured while still in bed in the morning, and sleep quality was assessed using a mat-type device. During the study period, the aromatherapy group used lavender or sweet orange aromatherapy throughout the night while sleeping, while the non-aromatherapy group was instructed to not use aromatherapy while sleeping. The total sleeping time and amount of rapid eye movement (REM) sleep differed significantly between the aromatherapy and non-aromatherapy groups ($p<0.01$). Differences were also observed in basal body temperatures during the ovarian follicular, corpus luteum, and menses phases of the menstrual cycle; however changes observed during the menses phase were not significant The amount of time spent in REM sleep in the aromatherapy group differed significantly between the ovarian follicular and corpus luteum phases ($p<0.01$), with the ovarian follicular phase also having an extended total sleep time ($p<0.05$). These results show that the effects of aromatherapy on sleep quality may be different during different phases of the menstrual cycle.

(COI: No)

P2-285

Sperm acrosomal membrane complex analyzed by STED using Equatorin-EGFP transgenic mice

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Background: The acrosome is a sperm organelle enclosed by a continuous acrosomal membrane, which is ultrastructually divided into the outer acrosomal membrane (OAM) and inner acorosomal membrane (IAM). The OAM and IAM make complexes with surrounding matrices containing molecules necessary for fertilization (the complex of acrosomal membranes and associating matrices: CAMAM). The CAMAM has not been visualized in detail under living condition due to the lack of good marker molecules for chasing the subtle changes and the resolution limit of the light microscope.
Purpose: In this study, using Eqtn-EGFP transgenic (Tg) mice and super-resolution stimulated emission depletion (STED) microscopy we analyzed the CAMAM.
Methods and Results: The images of CAMAM of Eqtn-EGFP Tg elongated spermatids taken by high-resolution microscopy, confocal laser scanning microscopy and STED microsocopy, compared with those of immunoelectoron microscopy. The CAMAM, which is generally analyzed at the light microscopy level, was further differenciated into its sub-components corresponding to the OAM-related and IAM-related stuructures (COAMAM and CIAMAM) at the nanometer scale in a whole sperm without sectioning by STED microscopy.
Conclusion: The information in this study will help for understanding the molecular mechanism of fertilization under living condition.

(COI: No)

P2-286

Histological investigation of impaired spermatogenesis in xeroderma pigmentosum group A gene (*Xpa*)-deficient mice

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Xeroderma pigmentosum (XP) has a defect in the initial step of nucleotide excision repair (NER) and consists of seven genetic complementation groups (groups A-G). XP group A patients have a high incidence of UV-induced skin tumors, immature testicular development, and neurological symptome. We reported that xeroderma pigmentosum group A (*Xpa*) gene-knockout mice [*Xpa* (-/-) mice] were deficient in NER and highly sensitive to UV-induced skin tumorigenesis. We found that the testis diminished in an age-dependent manner, and degenerating seminiferous tubules with vacuoles and no sperm were observed in the 24-month-old *Xpa* (-/-) mice. In this study, we investigated degenerating seminiferous tubules of *Xpa* (-/-) mice testis by immunostaining for autophagy-related proteins. We will discuss the implications of autophagy-related proteins immunostaining pattern for vacuole formation in *Xpa* (-/-) mice testis.

(COI: No)

P2-287

Aberrant formation of synaptonemal complex induced by Dnmt1 knockdown with in vivo electroporation of shRNA expression vector in mouse testes

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Mammalian spermatogenesis is an orderly arranged process consisting of spermatogonial proliferation, spermatocytic meiosis and spermiogenesis. Epigenetic factors, such as DNA methylation are thought to be involved in this process and the dynamic changes of methylation levels of CCGG sites were observed during mouse spermatogenesis by HELMET method. Therefore, in the present study, we analyzed the effect of Dnmt1 knockdown in spermatogenesis by in vivo electroporation of shRNA expression vector. LacZ shRNA expression vector was used as a negative control. The expression vectors were electroporated in 15 days-old mouse testes at the condition of 6 square 50 V electric pulses. Mice were sacrificed at 9 days after electroporation and testes were fixed overnight with 4% PFA in 0.01 M PBS (pH 7.4) and embedded in paraffin. The expressions of Dnmt1, 5-methylcytosine (5mC) and synaptonemal complex protein 3 (SCP3) were analyzed by immunohistochemistry. Dnmt1 was strongly expressed in spermatocytes of LacZ shRNA transfected testes and the expression levels of Dnmt1 and 5mC were decreased to 40% and 60% in Dnmt1 shRNA transfected spermatocytes, respectively. Apoptotic cells were increased to 240% and aberrant synaptonemal complex was observed in pachytene spermatocytes. These results suggest that maintenance of 5mC by Dnmt1 plays an essential role in the progression of meiosis through adequate formation of synaptonemal complex.

(COI: No)

P2-288

Goshajinkigan completely recover the severe aspermatogenesis after busulfan treatment in mice

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Busulfan is used as anticancer chemotherapeutic drugs in childhood and adult chronic myelogenous leukemia as well as an immunosuppressive agent before bone marrow transplantation. It is well known that the male infertility including spermatogenesis disturbance is one of side effect. There is little information about therapeutic drugs on male infertility after busulfan treatment. In the present study, we gave goshajinkigan to mice already having severe aspermatogenesis after busulfan treatment to determine whether or not the goshajinkigan can recover the aspermatogenesis. Male C57BL/6j mice were received a single intraperitoneal injection of busulfan at 4-week-old and after 60 days fed on the goshajinkigan-including diet or goshajinkigan-free normal diet for another 60 days. The results showed that after busulfan treatment, the progressively decreases in the weight of the testes (TW) and epididymal sperm count (ESC) in normal diet group from 60 days to 120 days; on the other hand, in goshajinkigan-including diet group, the dramatic recovery of these variables at 120 days, which is similarity to the normal spermatogenesis. These results suggest that busulfan-induced aspermatogenesis was irreversible unless receiving any medication. However, the supplementation of goshajinkigan can completely recover the regeneration of the injured seminiferous epithelium, suggesting that goshajinkigan have a therapeutic effect on busulfan-induced aspermatogenesis.

(COI: No)

P2-289

Identification of 5-bromo-2'-deoxyuridine-labeled cells during mouse spermatogenesis by use of a heat-induced antigen retrieval in lectin- and immunohistochemistry

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DNA replication occurs in the S phase of spermatogonia and preleptotene spermatocytes during spermatogenesis. BrdU is incorporated into synthesized DNA and is detectable in the nucleus by immunohistochemistry. To identify BrdU-labeled spermatogenic cells, the spermatogenic stages must be determined by visualizing acrosomes and the cell type-specific marker molecules must be detected in the seminiferous tubules. However, the antibody reaction with BrdU routinely requires the denaturation of DNA, which is achieved by pretreating tissue sections with hydrochloric acid; however, this commonly interferes with further histochemical approaches. Therefore, we examined optimal methods for pretreating paraffin sections of the mouse testis to detect incorporated BrdU by an antibody and, at the same time, visualize acrosomes with peanut agglutinin (PNA) or detect several marker molecules with antibodies. We found that treatment with heat-induced antigen retrieval (HIAR) consisting of heating at 95C in 20 mM Tris-HCl buffer (pH9.0) for 15 min was superior to that with 2N hydrochloric acid for 90 min at room temperature in the subsequent PNA-lectin histochemistry combined with double IHC for BrdU and one of the marker proteins. With this method, we identified BrdU-labeled spermatogenic cells during mouse spermatogenesis as A1 spermatogonia through to preleptotene spermatocytes.

(COI: No)

P2-290

Age-related changes of the wave of the seminiferous epithelium in rodent testes

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The wave of the seminiferous epithelium (SE) of the seminiferous tubules was assessed using 10-, 30-, 60-, and 90-week-old mice. The PAS-stained serial sections of testes were photographed, 3D-reconstructed in a computer to describe the wave of the SE. The typical complete wave, in which the all stages of the cycle of the SE line sequentially and it repeats along the length of the tubule, was observed only in the 10-week-old mice. In 30-week-old and older animals, the wave frequently reverses at the turning points of the winding tubules and the direction of the wave tends to be same in most tubule fragments. The rate of stage change in the unit length of SE becomes higher in older animals, meaning closer wave of seminiferous epithelium. At 90-week-old mice, the frequency of discontinuous wave significantly increased. Also increased the frequency of single cross sections of the tubule bearing two and more distinct stages of SE. These findings may correlate with decline in efficiency of sperm production in aged animals.

(COI: No)

P2-291

Left-right asymmetry of testicular formation in the chicken embryo

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Although all male vertebrates that use sexual reproduction have testes and the females have ovaries, the mechanism of gonadal formation differs among vertebrates. Most avian species including the chicken develop an ovary only on the female's left side. Testicular development in male chickens has been thought to be symmetric, but here we show that the testicular formation mechanism is asymmetric. We detected DMRT1, the most likely candidate transcription factor as a testicular determinant in the chicken, in not only Sertoli cells in seminiferous cords but also in cells constituting the cortex in the left gonad, whereas AMH, used as a Sertoli cell marker, was not expressed in the cortex. The cortex was gradually degenerated with testicular development, but interestingly, the serial localization of the cells expressing DMRT1 between the cortex and seminiferous cord was observed within a short period after sex determination. The localization of laminin and fibronectin illustrated the attachment of the seminiferous cord to the cortex. Recent studies indicated that Sertoli cells are derived fromnephrogenous mesenchyme in the chicken, but from coelomic epithelium in the mouse. However, the present findings show that only after sex determination and only in the left testis, the cells in the cortex apparently migrate into the seminiferous cord and contribute to Sertoli cells.

(COI: No)

P2-292

Cervical heterotopic transplantation technique of testis and epididymis

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The heterotopic transplantation techniques have been widely used in rat organs, such as kidney and heart, to investigate transplant immunology. On the other hand, the testis and epididymis are known as immunologically privileged organs. To determine the transplant immunology of testis and epididymis, we examined to establish a novel technique of rat testis and epididymis transplantation.

In the present technique, the testis and its artery and vein were isolated with the abdominal aorta and vena cava near the joint of testicular vessels. The anterior ends of removed vessels were ligated and the posterior ends were anastomosed to the common carotid artery and external jugular vein to maintain blood flow. The operations were performed in a syngeneic model, and two allogeneic models (= acute rejection model and chronic rejection model). The testes and epididymises were sampled after 3 or 7 days and the weights, histology and immune reactions of testes in each group were observed.

No weights nor histological changes in donor testes were observed in the syngeneic model and also in chronic rejection model neither at 3 days nor at 7 days. However, in the acute rejection model, spermatogenic disturbances were observed in testes at 3 days and undergoing necrotic changes were detected in testes at 7 days. These results were similar to those reported in heterotopic transplant of kidney and heart.

In conclusion, the newly developed operative procedure appears reliable for investigation of further heterotopic transplantation of the testis.

(COI: No)

P2-293

Quantitative analysis of the cellular composition in seminiferous tubules in normal and genetically modified infertile mice

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The aim of this study was to establish a quantitative standard of the cellular composition in seminiferous tubules at each stage of spermatogenesis in the mouse testis, and thereby evaluate abnormalities in the infertile mouse testis. We applied a combination of lectin histochemistry for acrosomes and immunohistochemistry for various specific cell markers, both visualized with fluorescence, on paraffin sections of the testis. We first examined seminiferous tubules from normal mice and counted the number of each cell type at each stage of spermatogenesis. We then examined seminiferous tubules from genetically modified mice deficient (-/-) for one of the cell adhesion molecules, nectin-2 or nectin-3, which were infertile, and compared the number of each cell type at each stage of spermatogenesis with the corresponding value in normal mice. In both nectin-2-/- and nectin-3-/- mice, despite the apparently normal morphology of the seminiferous epithelia, the later step spermatids were lost progressively, with the numbers of step 11-16 spermatids in nectin-3-/- and step 15-16 spermatids in nectin-2-/- mice being significantly lower than those in normal mice. The present study demonstrated that a quantitative analysis of cellular compositions at different stages in seminiferous tubules was useful for evaluating abnormalities in spermatogenesis.

(COI: No)

P2-294

The 3D-structure analysis of spermatids and the Sertoli cell by serial block face-SEM method

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Cells in biological tissues contact each other 3-dimensionally, and they have various interactions. In the seminiferous epithelium, the spermatogenesis is observed from the spermatogonium to the sperm, and these cells also contact Sertoli cells, suggesting that they have interactions. To understand these interactions, it is important to clarify 3-dimensional connections of the cells. However, for this purpose, TEM level spatial resolution is needed, since the cells are still connected by fine intercellular bridges even after cell division. In addition, analysis of several tens of cells is needed to understand whole 3-dimensional structure, since the spermatogonium undergoes several cell divisions during differentiation into the sperm. Thus, it is difficult to use TEM tomography and confocal laser scanning microscopy for this purpose. To overcome these difficulties, we applied a serial block face-SEM method to analyze the 3-dimensional structure of spermatids and Sertoli cells. In this method, an ultramicrotome is installed in the specimen chamber of a SEM, and the surface of a resin embedded specimen is cut at a predetermined thickness. Then the SEM image of the exposed specimen surface is captured. By repeating this process and stacking the captured SEM images, it is possible to reconstruct the 3-dimensional cell structure in high resolution in a large area. We took about 1000 SEM images of the sliced seminiferous epithelium. We recognized that there were 16 connected spermatids and the spermatid had 4 intercellular bridges. We report the detail in this presentation.

(COI: No)

P2-295

Lectin-binding sites in epithelial cells of the mouse prostate

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Prostate is an exocrine gland in the male reproductive tracts. The prostatic epithelium consists mainly of luminal and basal cells. Although prostate tumors are believed to originate in these cells, the mechanism of normal prostatic epithelial differentiation remains unclear. To understand the cytochemical properties of prostatic epithelial cells, the characteristics of glycoconjugates in the mouse prostate were examined using the technique of lectin histochemistry combined with immunohistochemistry. Characteristic staining patterns depending on the type of lectins were observed in the prostatic epithelium. Luminal cells expressed Mannose in all regions of the prostate, and Galactose, N-acetyl-D-galactosamine (GalNAc), and N-acetyl-D-glucosamine (GlcNAc) in the lateral and ventral regions. Interestingly, luminal cells in the ventral region specifically reacted with Jacalin lectin. Basal cells expressed GlcNac in the apical and dorsal regions of the prostate. These results indicate that the selectivity in lectin reactivity for distinct cell types and segment-dependent staining in the prostate may be related to cellular and regional differences in function. Furthermore, because some lectins stain particular prostatic epithelial cells selectively, these lectins could be useful markers for histopathological evaluation of diseases or diagnosis of male infertility.

(COI: No)

P2-296

Fatty acid binding protein 3 (FABP3) regulates PUFA transfer through trophoblast cells

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OBJECTIVES: Deficiency of polyunsaturated fatty acid (PUFA) transport in prenatal stage has been suggested to result in various adult metabolic diseases. In this study, we examined the localization and functional significance of fatty acid binding proteins (FABPs) in the mouse placenta.
METHODS: Expression of FABPs in the mouse placenta was examined by RT-PCR, western blotting and immunohistochemistry. Radio-labeled FA were administrated into the pregnant mother and its transfer to the fetus was measured by liquid scintillation counter. FA uptake assay was also done on FABP3 knockdowned (KD) BeWo cells. Placental morphology was examined.
RESULTS: In RT-PCR and western blotting, gene and protein expression of FABP3, 4 & 5 were detected in the mouse placenta with temporal differences. In immunohistochemistry, FABP3 was highly localized in the labyrinthine zone of mouse placenta; FABP4 was highly localized in the decidua basalis; FABP5 was weakly and widely distributed in the labyrinthine, decidua and spongiotrophoblast zone. In FABP3KO placenta, transportation of n-3 and n-6 PUFA was significantly decreased compared to wild-type. Consistently, FABP3 KD BeWo cells showed lower PUFAs uptake than control cells.
CONCLUSION: FABPs were expressed in the mouse placenta with spatial differences. Among FABPs, FABP3 may be involved in regulation of cellular transport of PUFAs, possibly being associated with various metabolic/psychiatric diseases caused by the fetal nutritional deficiency.

(COI: No)

P2-297

Different cell death induced in distinct breast cancer subtypes after drug treatment

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Multiple subtypes are including in breast cancer that has different genetic background, drug sensitivity and prognosis. For these features, subtype specific therapy is absolutely required. Recently, we reported that α-Mangostin, one of xthantones isolated from pericarp of mangosteen fruit, can induce apoptosis in highly malignant triple negative(TN) breast cancer cell line (HER2-, ER-, PgR-) and has antitumor activity in a mouse mammary cancer model. In this study, we investigated whether there are significant differences in induced cell death on distinct subtypes of human breast cancer cell lines after α-Mangostin treatment. We used triple negative breast cancer cell line MDA-MB231 and triple positive (TP) cell line MCF7 (HER2+, ER+, PgR+). After treatment, both cells showed significantly decreased viability until 24 hours. DNA damage and apoptosis are induced earlier in TN cell, compared to TP cell. In TN cell, mitochondria-mediated apoptosis was observed in contrast to TP cell. In TP cell, heat shock protein (HSP) are possibly mediate apoptosis because of its increased expression after treatment. To further elucidate the difference, we demonstrate the ultrastructural analysis via transmission electron microscopy.

(COI: No)

P2-298

Epithelium-dependent periodical excitation in response to stretch of guinea pig seminal vesicle

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Seminal vesicle (SV), a male accessory sex gland, is well-known to contract via activation of the sympathetic nerves. In addition to the nervous activity-dependent mechanism, we have found that epithelium of SV generates stretch-sensitive contraction. To explore further mechanisms of the epithelium-dependent contraction, we examined the effects of removal of epithelium from guinea pig SV on isometric contraction and membrane potential of the circular muscles. Epithelium-intact ring preparations contracted periodically at a frequency of 5.3 ± 0.3 /min at 36 ℃. The periodical contractions was abolished by 3 μM of nifedipine, suggesting they are associated with periodical activation of L-type Ca^{2+} channels. Removal of epithelium abolished the spontaneous contraction while nerve-evoked contraction was not impaired. To measure the membrane potential from the circular muscle layer, the tissue of SV (5 to 6 × 2 to 3 mm) was stripped of the serosa, longitudinal and outer circular muscle layers by dissection. In the presence of epithelium, the preparations could contract every 10 - 20 s by stretch. Six of nine cells in four epithelium-intact preparations, but none of the 16 cells in 12 epithelium-free preparations, exhibited periodical depolarization at 15.4 ± 0.4 s after the previous depolarization finished. These results suggest that the membrane oscillation in smooth muscles of SV may be produced by cells in the epithelial layer in response to stretch.

(COI: No)

P2-299

Reproductive and behavioral effects of clothianidin in male mice in a chronically stressed condition

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Neonicotinoids, which were developed in the 1990s, have been some of the most widely used pesticides in the world. They act as agonists to the nicotinic acetylcholine receptors (nAChRs) of insects with much higher affinity than to those of mammals, resulting in the death of insects from abnormal excitability in the cholinergic nervous system. However, as honeybee colony collapse disorder is suspected to be caused by neonicotinoids, there is rising concern about other unpredictable adverse effects of neonicotinoids on vertebrates such as birds and mammals. We hypothesized that the effects of neonicotinoids would be clear under chronic stress, which alters the expression of neuronal nAChRs. We performed immunohistochemical and behavioral analyses in male mice actively administered the neonicotinoid clothianidin (CTD) for 4 weeks under an unpredictable chronic stress procedure. We observed vacuolated seminiferous epithelia and decreased antioxidant enzymes, including glutathione peroxidase 4 and manganese superoxide dismutase, in the testes of the CTD-treated mice. In an open field test, locomotor activity and anxiety-like behaviors appeared to increase most in the CTD + stress mice. In summary, CTD and stress may additively affect the reproductive and behavioral functions of mammals.

(COI: No)

P2-300

Electro-acupuncture at sacral region enhances erectile function via central nerves system

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Electro-acupuncture (EA) influences various visceral functions via somato-autonomic reflex. We reported that EA at sacral region occurred increasing intracavernous pressure (ICP) by released nitric oxide from cavernousal nerve. The present study aimed to investigate whether ICP responses by EA at sacral region are mediated via central nervous system. Experiments were performed in isoflurane anesthesia rats. A catheter was inserted into the right carotid artery for blood pressure (BP) monitoring. ICP was measured with probe inserted to corpus cavernosum. EA was delivered by acupuncture needle, which inserted up to periosteal around 3rd foramina sacralia dorsalia, for 60 sec in a pulse width of 0.5 msec at a frequency of 10 Hz with an intensity of 5.0 mA. The spinalization was performed at the 13th thoracic after the EA response obtained, and then EA response was also confirmed at the same electrical condition. ICP was significantly increased by EA with an intensity of 5.0 mA, although BP was decreased. Furthermore, increasing ICP by EA was completely abolished by spinalization. These results demonstrate that EA at sacral region enhances erectile function via central nervous system. In conclusion, EA may be usefulness on the erectile dysfunction via central nerves system.

(COI: No)

P2-301

Partial blockade of Kv2.1 channel potentiates GLP-1's insulinotropic effects in islet β-cells and improves glucose tolerance in type 2 diabetes

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Glucagon-like peptide-1 (GLP-1)-based medicines have been widely used to treat type 2 diabetic patients. Inhibition of voltage-gated Kv2.1 channels in pancreatic β-cells is suggested to contribute to mild depolarization and promotion of insulin release. This study aimed to determine whether blockade of voltage-gated Kv2.1 channels potentiates insulinotropic effect of GLP-1. Kv2.1 channel blocker guangxitoxin-1E (GxTx) and GLP-1 agonist exendin-4 at sub-threshold concentrations, when combined, markedly increased insulin release and cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in a glucose-dependent manner in mouse islets and β-cells. Exendin-4 at sub-threshold concentration alone increased islet insulin release and β-cell $[Ca^{2+}]_i$ in Kv2.1$^{+/-}$ mice. The β-cell $[Ca^{2+}]_i$ response to sub-threshold exendin-4 and GxTx in combination was attenuated by protein kinase-A inhibitor H-89. Kv2.1$^{+/-}$ mice exhibited improved glucose tolerance and increased plasma insulin levels during oral glucose tolerance tests that promote endogenous GLP-1 release, compared to wild-type mice. Furthermore, administration of sub-threshold doses of GxTx and GLP-1 agonist liraglutide in combination markedly increased plasma insulin and improved glucose tolerance in diabetic db/db mice and NSY mice. These results demonstrate that a modest suppression of Kv2.1 channels dramatically raises insulinotropic potency of GLP-1-based drugs, providing a potential therapeutic tool to treat type 2 diabetes.

(COI: No)

P2-302

Differential responses to steroid hormones in fibroblasts from the vocal fold, trachea and esophagus

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Fibroblasts are target cells for steroids such as sex hormones and corticoids. The characteristics of fibroblasts vary among tissues and organs. We compared the action of steroid hormone on cultured fibroblasts from the vocal folds, which are considered to be the primary target of steroid hormones, as well as the trachea and the esophagus in adult male rats. Expression of steroid hormone receptors (androgen receptor (AR), estrogen receptor α, and glucocorticoid receptor) was confirmed by immunofluorescence detection. AR was more frequently expressed in the vocal fold fibroblasts than in the tracheal and esophageal fibroblasts. Cell proliferation analysis exhibited that either administration of testosterone (T), estradiol (E2), or corticosterone (CORT) suppressed cell growth in all three fibroblasts. mRNA expression of extracellular matrix-associated genes represented that the addition of T, but not of E2 or CORT, markedly promoted the expression of procollagen I and III, elastin and hyaluronic acid synthase I only in the vocal fold fibroblasts. These results indicate that each steroid hormone exerts region-specific effects on cervicothoracic fibroblasts with different properties through binding to each receptor. These findings might help clarify the mechanism of voice change and mutational voice disorder during puberty.

(COI: No)

P2-303

Identification of novel estrogen receptor α variants in the human and mechanism of transcriptional activation

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The human ER α gene is composed of eight coding exons, and generates several splice variants. However, it remains to be fully elucidated. Therefore, we decided to identify other human ER α splice variants and to re-examine the genomic organization of the human ER α gene. We cloned novel C-terminally-truncated variants using rapid amplification of cDNA 3'-ends, and identified novel terminal exons and novel alternative splice acceptor sites. Subsequently, we comprehensively analyzed the distribution of human ER α variants in the human peripheral organs using reverse transcription-polymerase chain reaction. The variant mRNAs were detected in a wide range of organs. Subsequently, we constructed expression vectors encoding wild-type, novel C-terminally truncated and artificially truncated ER α proteins, and characterized subcellular localization and transactivation functions of the variants in transfected cells. Moreover, we identified the mechanism of transcriptional activation that is ascribable to the structure of helices 3 to 5 in the ligand binding domain of ER α. In this study, we demonstrated that the ER α gene generates C-terminally truncated variants with distinct localization patterns and functions by alternative usage of intronic exons, and that the helix structure contribute to the transcriptional activation of the ER α protein. These findings provide useful information for further investigation on estrogen related physiological and pathophysiological processes.

(COI: No)

P2-304

Role of IL-6 in metabolic abnormalities of streptozotocin-induced diabetes

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We have found that inhibition of AMPK activity in skeletal muscle in STZ-induced diabetes by preferentially expressing dominant-negative AMPK (DN-AMPK) significantly improved STZ-induced hyperglycemia, and high level of plasma free fatty acids and ketone bodies, although plasma insulin level was low. Moreover, STZ-treated DN-AMPK mice improved atrophy of white adipose tissue (WAT) and skeletal muscle, body weight loss and increased survival rate. Chronic infusion of AMPK inhibitor, compound C, also improved the metabolic abnormities in spontaneously developed non-obese diabetic (NOD) mice as well as STZ-induced diabetes.
In this study, we investigated a role of IL-6 (Interleukin-6), a myokine secreted from skeletal muscle in STZ-induced diabetes. STZ-induced diabetes increased IL-6 protein expression level in skeletal muscle and plasma IL-6 level, and those were returned to the control levels in DN-AMPK mice. IL-6 protein expression was not changed in other tissues, such as liver, adipose tissue or spleen. The downstream factor of IL-6 signal, STAT3, was elevated in soleus muscle, WAT and BAT in STZ-induced diabetes, but this change was abolished in STZ-treated DN-AMPK mice. Infusion of neutral antibody for IL-6 by osmotic minipump improved the metabolic changes and survival rate in STZ-induced diabetes, similar to those in STZ-treated DN-AMPK mice. These results thus unveil a key role for muscle AMPK and IL-6 in metabolic abnormities in STZ-induced diabetes.

(COI: No)

P2-305

E-cadherin mediates Notch signaling in the rat anterior pituitary

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Anterior pituitary of the rat consists of hormone-producing cells and folliculo-stellate (FS) cells. FS cells construct unique microenvironment by homophilic cell adhesion and be assumed to be progenitor cells of hormone-producing cells at least in part. We have shown that Notch signaling plays important roles to regulate proliferation activity and SOX2 expression of FS cells. Notch signaling belongs to the juxtacrine signaling that requires specific cell adhesion. In the present study, we aimed to examine the possibility that E-cadherin, a specific cell adhesion molecule of FS cells establishes the Notch signaling among FS cells. By immunohistochemistry using transgenic rats that express GFP specifically in FS cells, it is shown that Notch2 and jagged1 are major receptor and ligand in FS cells, respectively. They are shown to be expressed specifically in cell clusters with E-cadherin. SOX2 was expressed in these cell clusters. It is also shown that Notch 2 and jagged1 are expressed in the same cells, which strongly suggest that FS cells affect each other to maintain undifferentiated state. Taken together with our previous report that cadherin isoforms switch from E-cadherin to N-cadherin when embryonic progenitor cells of anterior pituitary differentiate into hormone producing cells, results of the present study may suggest that E-cadherin construct a microenvironment for presumptive progenitor cells in the adult anterior pituitary to maintain undifferentiated state by Notch and other signaling.

(COI: No)

P2-306

Extracellular matrix actions in rat anterior pituitary gland: I. Its effect on hormone release from gonadotrophs

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The anterior pituitary gland is composed of five types of hormone-producing cells, folliculostellate cells, endothelial cells, pericytes, and the various extracellular matrixs (ECMs). The ECM supports adhesion of cells and is also important for cell survival, proliferation, differentiation, and migration via its receptors (integrins) in various tissues. Hormone-producing cells and folliculostellate cells form lobules that are surrounded by ECM in rat anterior pituitary gland. In the lobular structure, adhesion of hormone-producing cells and folliculostellate cells to basement membrane is observed and these cells are close to collagen fibril. We previously reported the cells that produce ECM components including collagen, laminin and proteoglycan in rat anterior pituitary gland. However, there is little information about effect of ECM on hormone release from the adenohypophyseal cells. In this study, we investigated whether ECM affect to luteinizing hormone (LH) release using male rat anterior pituitary cells. We compared hormone secretion from cultured cells on ECM-coated plate with that of on non-coated plate in various experimental conditions. LH release level from cultured cells on ECM-coated plate was decreased as compared with cultured cells on non-coated plate. This result suggests that ECM contribute to regulation of LH release from gonadotropes.

(COI: No)

P2-307

Rat uterine oxytocin receptor and estrogen receptor α and β mRNA levels are regulated by estrogen through multiple estrogen receptors

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In the rat uterus, oxytocin receptor (OTR) and estrogen receptor (ER) levels are regulated by estrogen; however, which types of ERs are involved have not been elucidated. This study examined OTR, ER α , and ER β levels in ovariectomized rats treated with: 17 β -estradiol (E2), the ER α agonist (PPT), the ER β agonist (DPN), the GPR30 agonist (G-1), and estren (Es). E2 and PPT increased OTR mRNA levels and decreased ER α and ER β mRNA levels 3 and 6 h post-treatment. DPN decreased ER α and ER β mRNA levels at 3 and 6 h, while OTR mRNA levels increased at 3 h and decreased at 6 h. After Es treatment, OTR mRNA levels increased at 3 h and then declined until 6 h, whereas ER α and ER β mRNA levels decreased by 3 h and remained low until 6 h post-treatment. G-1 had no effect on OTR, ER α , and ER β mRNA levels either at 3 or 6 h. The ER antagonist ICI182,780 (ICI) suppressed Es-induced increases in OTR mRNA levels at 3 h. However, neither ICI nor tamoxifen (Tam) had any significant effect on ER α and ER β mRNA levels in the Es-treated group. In intact rats, proestrus-associated increases in OTR mRNA levels were antagonized by ICI and Tam, but not by the GPR antagonist G15, while decreases in ER α were antagonized by ICI, but not by Tam or G15, and decreases in ER β were antagonized by Tam and G15, but not by ICI. Taken together, these results show that, in the rat uterus, expression of the OTR gene and of ER genes is regulated by estrogen through multiple pathways that involve Es-sensitive ERs and/or GPR30.

(COI: No)

P2-308

The mechanism of intracellular cAMP concentration increase induced by extremely low-frequency magnetic field exposure in mouse adrenal-derived Y-1 cell

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We previously reported that exposure of extremely low-frequency magnetic field (ELF-MF) induces adrenal steroid synthesis in mouse adrenal Y-1 cell line via increase of intracellular cyclic adenosine monophosphate (cAMP) concentration. However, precise mechanism of the cAMP increasing is unknown at present. The cAMP concentration is decided by the ratio of the cAMP synthesis by adenylate cyclase, which activates with Gs family of G-protein, and the degradation by phosphodiesterase (PDE). In this study, we investigates the effect of Gs α -subunit inhibitor NF449 on the increase of cAMP concentration by ELF-MF exposure and PDE activity in ELF-MF exposed cell to clarify the mechanism on the ELF-MF induced adrenal steroid synthesis in Y-1 cell. As the results, NF449 is decreased the cAMP concentration in sham and ELF-MF exposed Y-1 cell, but the cAMP-increasing effect of ELF-MF was preserved. On the other hand, PDE activity was decreased in ELF-MF exposed Y-1 cell. Our results suggest that the increase of intracellular cAMP concentration induced by ELF-MF exposure in Y-1 cell may be involved in the decrease of PDE activity not the activation of G protein-coupled receptors.

(COI: No)

P2-309

Relationship between perinatal hypothyroidism and structure/function of Purkinje cells

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Thyroid hormone (TH) is very important for increasing the basal metabolic rate of almost all cells to achieve the healthy function of organs. It is also important for brain development and maturation during perinatal period. Perinatal hypothyroidism induces anatomical and functional deficiency such as motor discoordination by cerebellum. The most problem thing is that some effects of TH have critical period and deficiency of perinatal short-term hypothyroidism are prolonged until adulthood. However, the relationship between the effect of hypothyroidism on functions of cerebellum and the anatomical and/or electrophysiological changes of cerebellum is remained unclear. In this study, we examine the effect of perinatal hypothyroidism on function and structure of Purkinje cell (PC) in cerebellum by using slice patch clamp technique. We observed the synaptic activity of parallel fiber -PC and climbing fiber-PC synapses and after the experiment, stained the PC with biocytin/streptavidin for anatomical observation. In addition, we investigated the quantity of proteins which are involved in the machinery of synaptic release at presynapses contained soluble N-ethylmaleimide-sensitive factor-attachment protein receptor (SNARE) proteins for hypothyroid mice because we previously reported the possibility of presynaptic dysfunction in congenital hypothyroidism mice (Amano et al., J. Physiol. Sci., S2210, 2014).

(COI: No)

P2-310

Learning deficits in a mouse model of perinatal mild hypothyroidism

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Thyroid hormone (TH) is essential for brain development. It is known that congenital hypothyroidism causes a wide spectrum of severe neurological deficiencies in rodents and human. It is also recently concerned that decrease of taking iodine diet or increase of environmental chemicals cause low level hypothyroidism. However, mild and/or moderate hypothyroidism on brain development is felly studied. Thus, we examined the behavior adult mice which induced mild hypothyroidism during perinatal period by using low dose propylthiouracil (PTU) application. We added the PTU (5 or 50 ppm) in drinking water for the mother mice and their pups from gestational day 14 to postnatal day 21. TH levels of pups were significantly decreased in both 5 and 50 ppm groups compared with those in control pups. However poor bodyweight gain was only shown in 50ppm group. Cognitive performances were assessed using novel object recognition test and novel object location test on postnatal 8 weeks. Clear differences were absent in both short (15 min) and long(24 hr) term memory. To detect discrimination learning, touch-panel discrimination test was performed on postnatal 9-10 weeks. Discrimination levels of 50 ppm group are lower than other groups on day 3-4. However same levels of discrimination index were shown in all groups on day 5-9. These data suggest that mild hypothyroidism partially prevents cognitive function and causes delay of learning according to disorders of neuroplasticity or neuronal circuit function itself.

(COI: No)

P2-311

Mechanism of proliferation of pancreatic beta cells in ventromedial hypothalamus-lesioned rats

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The ventromedial hypothalamus (VMH)-lesions induce hyperplasia of the abdominal organs via hyperactivity of the vagal nerves. Recently betatrophin that is a liver-secreted hormone induces pancreatic β cell proliferation. The aim of this study is to clarify the mechanism of cell proliferation in the pancreas of the VMH-lesioned rats using histological and immunohistochemical techniques and measurement of the gene expression level of betatrophin in the liver. The bilateral VMHs of female Sprague-Dawley rats were electrically lesioned. Specimens were prepared at 5 days after the operations. The expression level of betatrophin mRNA in the liver was measured using a real-time PCR. Mitotic cells were observed in the pancreatic islet of VMH-lesioned but not sham-operated rats. Double immunostaining revealed that Ki-67 was localized in the insulin-immunopositive cells. Mitotic cells consisted mainly mature β cells with small part of undifferentiated cells at electron microscopic level. The gene expression level of betatrophin was significantly higher in VMH-lesioned than in sham-operated rats. These results suggested that proliferation of β cells by VMH lesions was mainly caused by self-replication mechanism. Betatrophin may contribute to self-replication of β cells in VMH-lesioned rats.

(COI: No)

P2-312

Specific localization of zinc transporters and zinc-required proteins in the pancreatic islet of rat

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The islet of rat pancreas is composed by 5 different endocrine cell types (A cell, B cell, D cell, PP cell, and E cell); the B cell occupies the central portion, the others the peripheral portion. By the immunohistochemical studies, two zinc transporters (Znt5, ZIP7) and 3 zinc-required enzymes, Carbonic Anhydrase (CA) II, XII, and Carboxypeptidase (CP) A localized in the peripheral portion of the islet. To identify their origin to the specific endocrine cell type, double immunofluorescence labeling was performed using the antibodies of zinc-related proteins and those of glucagon (GCG), somatostatin (SST) and pancreatic polypeptide (PPY). The frozen sections of normal and zinc-treated rats (ZnSO4 5mg/100g.b.w. i.p. one injection /day for 2days) were subjected to immunofluorescence labeling. The labeling showed, CAII was co-localized with some of PPY-positive cells, but neither with GCG-, nor SST-positive cells. Those of ZnT5, ZIP7, CAXII, CPA were co-localized with GCG-positive cells, but not with SST-positive cells. Although the B cell in the islet is known to contain much zinc for crystallization of insulin molecules in the secretory granules, significance of zinc in the A cell has not been well understood. In this experiment, many zinc-related proteins were specifically localized to the α cell in the islet, but not to the other cell types. The significance of zinc in the A cell will be discussed, considering the specific structural organization of the pancreatic islet.

(COI: No)

P2-313

Continuous GnRH signal affects the ultrastructure of endomembrane systems in male rat pituitary gonadotropes

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Gonadotropin synthesis and secretion are regulated by GnRH signaling. To examine whether continuous GnRH signals affect the morphological characteristics of gonadotropes, we analyzed the ultrastructural and immunocytochemical changes in endomembrane systems of male rat pituitary gonadotropes during sustained treatment with various GnRH agonists. In gonadotropes at 1 day after subcutaneous implantation of osmotic pumps filled with GnRH agonists, leuprorelin or buserelin, patch-like accumulations of chaperons such as calnexin and BiP appeared. By electron microscopy, the ER chaperones were accumulated in the anomalous network of tubuloreticular membranes within the stimulated gonadotropes. To these atypical tubuloreticular membranes, an E3 ligase HRD1 involved in the ER associated degradation (ERAD), was colocalized with the chaperones. Simultaneously, or slightly behind the time, multi-lamellar autophagosome-like structures occasionally appeared in the cytoplasm of gonadotropes containing the ER patches. These findings suggest that continuous GnRH signals induce dynamic reorganization of endomembrane systems related to the intracellular protein degradation.

(COI: No)

P2-314

β-Cell specific *Mafk* Overexpression Impairs Pancreatic Endocrine Cell Development

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Pancreatic β-cells are the only cells that can secrete insulin to maintain normal glycaemia. The MAF transcription factor proteins are homologs of v-MAF, the oncogenic component of the avian retrovirus AS42. We previously found that β-cell specific overexpression of *Mafk* exhibited glucose intolerance in adulthood. The aim of this study is to examine the effect of β-cell specific *Mafk* overexpression in embryonic endocrine pancreas. The developing islets of transgenic embryos appeared disorganized with an inversion of insulin- to glucagon-positive cell ratio at both E15.5 and E18.5. Moreover, the total insulin content significantly decreased in transgenic embryos. Immunohistochemical analysis using Ki67 antibody showed a lower ratio of proliferating β-cells in the transgenic embryos compared to control embryos, suggesting that damaged β-cell proliferation resulted in the abnormal endocrine structure of transgenic islets. The examination of gene expression profiles by Q-PCR revealed that *insulin* genes were significantly decreased accompanied with the reduction of several β-cell related genes including *Slc30A8/*ZnT8, *Npy*, and *G6pc2*. In contrast, both transcription factors essential for β- and α-cell development including *Pax4*, *Nkx2.2*, *Arx*, and *Mafb* were upregulated. Our results suggested that β-cell specific *Mafk* overexpression impaired embryonic endcrine development, and to further examination of this model might be useful for the better understanding of the molecular basis that govern β-cell development.

(COI: No)

P2-315

Calcium imaging of vasopressin neuron in the hypothalamic culture derived from mouse embryonic stem cell

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Recently, mouse embryonic stem cell has been successfully induced to hypothalamic tissue that express variety of hypothalamic neuropeptides. The feederless mES line, EB5, quickly aggregates to form embryoid body in serum-free medium in the phospholipid-coated well (SFEBq). In growth factor-free chemically defined medium (gf-CDM), it differentiates to Rax^+ hypothalamic progenitor cells in SFEBq that further mature to hypothalamic neurons including $vasopressin^+$ neuron. For physiological investigations, we have engineered the vasopressin::GFP cell line ($AVP^{GFP/+}$) from $Rax^{GFP/+}$ mESC using TALEN nuclease system. Hypothalamic progenitor cells expressing Rax were purified by FACS-sorting, and cultured on cover glass for further maturation. A week later, $AVP^{GFP/+}$ cells can be identified by fluorescence microscope and the expression of AVP was identified with immunocytochemistry. On the calcium imaging analysis using Fura2-AM, Glutamate increased $[Ca^{2+}]_i$ and GABA attenuated the glutamate-induced response in the $AVP^{GFP/+}$ cells. In some of the $vasopressin^+$ neuron, glutamate response was suppressed by the addition of NERP-1, which supposed to be a dendritic released transmitter from vasopressin neuron. These findings suggest this system would contribute to elucidate the detailed mechanism of vasopressin release.

(COI: No)

P2-316

Immunoelectron microscopic study on the subcellular localization of kisspeptin, neurokinin B and dynorphin A in KNDy neurons of the female rat

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KNDy neurons in the hypothalamus are coexpressing three kinds of neuropeptide, kisspeptin, neurokinin B (NKB) and dynorphin A (DynA), and are known to be associated with generation of GnRH / luteinizing hormone (LH) pulse to control follicular growth and steroidogenesis. However, the subcellular localization of these neuropeptides in KNDy neurons are not clear. In this study, we analyze the subcellular localization pattern of three neuropeptides using the immunoelectron microscopy. The female rat brain were fixed, dehydrated in ethanol and embedded in epoxy resin. The hypothalamic areas containing KNDy neurons was selected and trimed under stereoscopic microscope, then ultra thin sections were cutted. We performed the post-embedding immunoelectron microscopy with each antibody of kisspeptin, NKB, DynA, and visualized by colloidal gold-2nd antibody complex, and observed them using a transmission electron microscope. Three neuropeptides, kisspeptin, NKB and DynA were observed in each different secretory vesicle in KNDy neurons. We succeeded to reveal that three neuropeptides in KNDy neurons were contained in each different secretory vesicle, suggesting that these neuropeptides in the KNDy neurons were differentially regulated. Our present results will clearly contribute to consider the regulation mechanism of kisspeptin secretion in KNDy neurons, and the generation of GnRH / LH pulse induced by kisspeptin.

(COI: No)

P2-317

Molecular and histochemical analysis on acute modulation of the *Kiss1* expression in the lactating rat hypothalamus mediated by the suckling stimulus

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In female mammals, lactation suppresses GnRH secretion resulting in transient infertility. In rats, GnRH secretion is recovered in 18 h after pup separation (PS) and rapidly re-suppressed by re-exposure of pups. In order to elucidate the mechanisms underlying these modulations, changes in the expression of kisspeptin, a stimulatory modulator for GnRH secretion, were examined. 4h or 18h PS significantly increased *Kiss1* expression in both the anteroventral periventricular nucleus (AVPV) and the arcuate nucleus (ARC), and subsequent 1h exposure of pups re-suppressed *Kiss1* in the AVPV. Change in *Kiss1* expression was observed prior to the changes in GnRH, indicating that the changes in GnRH secretion result from the change of kisspeptin. We further examined the mechanisms of the rapid modulation of *Kiss1* expression. We firstly examined the effect of prolactin. Intravenous administration of prolactin suppressed *Kiss1* expression in the AVPV. We also examined the possibility that the suckling stimulus modulate the *Kiss1* expression through ascending sensory input. Injection of the anterograde tracer to the subparafascicular parvocellular nucleus (SPFpc) in midbrain which relay suckling stimulus revealed direct neuronal connections between the PSFpc and kisspeptin neurons in both the AVPV and ARC. These results indicate that suckling stimulus rapidly modulate *Kiss1* expression directly via neuronal connections, and partially through serum prolactin, resulting in modulation in GnRH secretion.

(COI: No)

P2-318

Morphological analysis of spines of the GnRH neuron through pubertal development

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The onset of puberty is initiated by augmentation of gonadotropin-releasing hormone (GnRH) release from GnRH neuron. However, the precise mechanism which leads the changes in the activity of GnRH neurons at the puberty onset is still unclear. The spine, small protrusions on the surface of neuronal dendrites, normally receives excitatory inputs. In this study, we analyzed the number of spines of GnRH neuron to determine the changes in synaptic inputs through puberty, using 3 and 8 weeks of age GnRH-eGFP transgenic rats. We also measured the diameter of head (DH) of each spine and classified them into small (DH<0.65 μm), large (DH0.65 μm), and giant spine (DH0.9 μm). The greatest number of spines was observed at the proximal dendrite (<50 μm from soma). At the soma and proximal dendrite, the number of spines was greater in adult than in juvenile in both sexes. Classification of spines revealed that the increases in large and giant spines at soma and the proximal dendrite. To further explore the relationship between the spines of GnRH neuron and puberty, we analyzed the adult rats neonatally exposed to estradiol benzoate, in which puberty onset and reproductive functions is disrupted. Neonatal estrogenization resulted in decreases in the number of all types of spines, so that total number of spines, at soma and dendrites in both sexes. These results suggest that GnRH neurons become to receive more and lager excitatory inputs on the soma and the proximal dendrite through puberty, and the changes in the spines play pivotal roles in the normal pubertal development.

(COI: No)

P2-319

Age-related alterations of KNDy neuron and pulsatile LH release in female rats

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After menopause, GnRH/Luteinizing hormone (LH) pulse frequency, which is essential for follicular growth, is decreased. Kisspeptin /Neurokinin B (NKB) /Dynorphin (KNDy) neuron in hypothalamic arcuate nucleus is known to be associated with GnRH/LH pulse generation, but the age-related changes in the expression of KNDy neuron and pulsatile LH release in female rats has not been examined.
In this study, we studied on this unsettled issue by using 8-16 week-old (young), 13, 20, and 24-month-old (13M, 20M, 24M) female rats. Blood samples were collected every 6 minutes for 3 hours to measure LH concentration by RIA. After fixation of rat brains, the coronal cryosections were prepared for in situ hybridization of *Kiss1* (Kisspeptin gene), *Tac2* (NKB gene), and *Pdyn* (Dynorphin gene) mRNA.
Plasma LH concentration was reduced during aging as previously reported. The expression of *Kiss1* was significantly decreased in 20M and 24M compared to young and 13M. Although both *Tac2* and *Pdyn* expression were also significantly decreased in aged animals compared to young, *Tac2* expression was maintained at relatively high level compared to *Pdyn* in aged animals.
These results suggest that each gene in KNDy neuron may be controlled by different signal pathway, and the reduction of KNDy expression may cause altered pulsatile LH secretion in perimenopausal period.

(COI: No)

P2-320

Functional significance of angiogenesis-associated vascular plasticity in neurosecretion of the neurohypophysis

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Hypothalamo-neurohypophysial system releases arginine vasopressin (AVP) and oxytocin (OXT) from axonal terminals of the neurohypophysis (NH) into blood circulation for controlling body fluid homeostasis and lactation. Chronic osmotic and suckling stimulations have been shown to cause neurovascular and neuroglial reconstruction in the NH of adult mammals and no study has been reported for vascular dynamics. The aim of this study was to elucidate the occurrence of continuous angiogenesis and growth factor-dependent neurovascular reconstruction in the NH of adult mice. Active proliferation of endothelial cells was observed using the immunohistochemistry of bromodeoxyuridine and Ki-67. Vascular endothelial growth factor A (VEGFA) and VEGF receptor 2 (VEGFR2) were highly expressed at pituicytes and endothelial cells respectively. Administration of the selective tyrosine kinase inhibitor AZD2171 for VEGFRs significantly decreased proliferation of endothelial cells. Moreover, AZD2171 treatment decreased vascular density by facilitating apoptosis of endothelial cells and the withdrawal of its treatment led to remarkable rebound proliferation of endothelial cells. AZD2171 decreased the density of both AVP- and OXT-containing axonal terminals. Thus, this study demonstrates that the signaling pathways of VEGF are crucial mediators for determining proliferation of endothelial cells and the density of AVP- and OXT-containing axonal terminals in the NH.

(COI: No)

P2-321

Identification of transcriptional and posttranscriptional regulation of human estrogen receptor expression in the testis

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Expression of estrogen receptors (ERs) are subject to complicated regulation. Transcription of the ER genes is initiated by multiple promoters. Furthermore, alternative promoter usage and alternative inclusion of untranslated internal exons yield multiple variants with distinct 5'-untranslated regions (5'-UTRs), which influence translational efficiency and mRNA turnover. Estrogens exert their effects via activation of two types of ERs (ER α and ER β), and have pivotal roles in the testis. However, several previous reports are inconsistent in expression patterns of ER α and ER β in the human testis. Therefore, we examined the expression and regulation of the human ER genes in the testis. RT-PCR analysis revealed that both ER α and ER β mRNAs are expressed in the testis. Then, we analyzed expression of promoter-specific ER isoforms to evaluate which promoters are selectively utilized in the testis. The expression of ER α mRNAs was initiated mainly by activation of F and T promoters. All promoter-specific ER β isoforms (0K, 0N, and E1 isoforms) were highly expressed. Subsequently, we assessed posttranscriptional regulation of the isoforms using luciferase reporter assays. The analysis demonstrated that the translation of ER α T isoforms was severely suppressed. These results indicate that expression of human ER α is repressed posttranscriptionally in the testis and that transcription of testicular ER β mRNA is initiated by strong activation of three promoters.

(COI: No)

P2-322

IL-1β produced by Microglia/macrophage in the organum vasculosum of the lamina terminalis is involved in the suppression by lipopolysaccharide of steroid-induced luteinizing hormone surge in ovariectomized rats

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Inflammatory/immune challenge is known to suppress luteinizing hormone (LH) secretion. We previously reported that pretreatment with minocycline (Mino), a potent inhibitor of microglial activation, significantly alleviate the suppression by lipopolysaccharide (LPS) of ovarian steroid-induced LH surge, but did not affect the increment of serum cytokines, treatment in ovariectomized (OVX) rats. In this study, we examined the effect of Mino on cytokines induction by LPS in the preoptic area of ovarian steroid-primed OVX rats. Mino or saline was administered intraperitoneally once a day for four consecutive days. LPS or saline was injected intravenously at noon. Brain fragments including the preoptic area were collected at 14.00 h, and gene expressions of IL-1β, IL-2 and IL-6 were measured by real-time PCR. Cytokine was also examined localization. IL-1β and IL-6, but not IL-2, gene expressions were increased by LPS treatment. Mino pretreatment significantly attenuated the induction of cytokines by LPS. IL-1β immunoreactivity was found only in Iba1 (a marker of microglia/macrophage) immunoreactive cell in the organum vasculosum of the lamina terminalis (OVLT) in LPS treated rats. These results suggest that IL-1β produced by microglia/macrophage in the OVLT might be involved in the inflammatory/immune challenge induced suppression of LH secretion.

(COI: No)

P2-323

Postnatal Changes of Distribution of S-100 Protein Positive Cells, Connexin 43 and LH-RH Positive Sites in the Pars Tuberalis of the Rat Pituitary Gland

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Folliculo-stellate cells are characterized by their star-like morphological features and specific ability to form follicles found within the adenohypophysis of rats. The architecture of luteinizing hormone-releasing hormone (LH-RH) nerve ends and the S-100 protein containing folliculo-stellate cells forming gap junctions in the pars tuberalis is basically important in understanding the regulation of the hormone producing mechanism of anterior pituitary glands. In this study, we investigated the sexual maturation of the anterior pituitary glands through the postnatal development of S-100 positive cells, connexin 43 and LH-RH nerves. Intact male rats 5 to 60 days old were prepared for immunohistochemistry, the S-100 containing cells in pars tuberalis were first detected on day 30 and increased in number to day 60; this was parallel to the immunohistochemical staining of gap junction protein, connexin 43. LH-RH positive sites were clearly observed on just behind the optic chiasm and on the root of pituitary stalk on day 30. On day 60, the width of layer increased, while follicles and gap junctions were frequently observed between agranular cells of pars tuberalis. It is suggested that the folliculo-stellate cell system including the LH-RH neurons in the pars tuberalis participates in the control of LH secretion along with the portal vein system.

(COI: No)

P2-324

Expression of vasopressin in rat peripheral tissues

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Vasopressin and oxytocin, neurohypophysial hormones, are nine-amino acid peptides which are best known for their classical abilities to increase peripheral vascular resistance by water reabsorption in the collecting ducts of the kidney nephron and by vasoconstriction, and contract smooth muscle of uterine and mammary gland, respectively. In addition, they seem to work in the central nervous system and other peripheral organs than described above. We have showed oxytocin distribution in the peripheral organs (2014, The 119th Annual Meeting of the Japanese Association of Anatomists). Then, we performed an analysis for vasopressin distribution in peripheral organs. Using real time RT-PCR technique, in addition to hypothalamus and posterior pituitary gland, vasopressin mRNA was expressed widely in anterior pituitary gland, pancreas, parotid gland, submandibular gland, heart and kidney. At the cellular expression level by immunohistochemistry, vasopressin immunoreactivity was seen in secretary duct epithelial cells in pancreas and salivary glands, in secreting cells of anterior pituitary gland and parotid gland, and in uriniferous tubules and podocyte. Majority of those cells also shows oxytocin immunoreactivity. However, cells in islets of Langerhans, which synthesize oxytocin, showed no vasopressin immunoreactivity. Results suggest that some peripheral tissues synthesize both vasopressin and oxytocin, and that vasopressin and oxytocin may interact together to control cells in a paracrine manner.

(COI: No)

P2-325

Effects of proton pump inhibitor on organs synthesizing estrogen in male rats

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Proton pump inhibitor (PPI) suppresses a gastric acid secretion of parietal cells of the stomach. It has been known that the parietal cells in rats synthesize a large amount of 17 β-estradiol. However, effects of PPI on their estrogen synthesis are unclear. Furthermore, the effects on the synthesis in the testis and adrenal gland, which are known as the main organs of the 17 β-estradiol synthesis in male, is also unclear. Twelve adult male Wistar rats were separated into the following three groups; LPZ1d: oral administration (OA) of lansoprazole (30 mg/kg) in 0.5% carboxymethylcellulose (CMC) solution for 1 day, LPZ3d: OA of lansoprazole in 0.5% CMC solution for 3 day, control: OA of 0.5% CMC solution. The 17 β-estradiol level of the blood in the portal vein and abdominal aorta was examined by ELISA. The aromatase mRNA and protein level of the gastric mucosa, testis, and adrenal gland were examined by real-time PCR, and by immunohistochemistry and Western blotting, respectively. The 17 β-estradiol level in the portal vein and abdominal aorta in LPZ3d was higher than that in the control. The aromatase mRNA and protein in the gastric mucosa were much enhanced in LPZ3d. The aromatase in the testis and adrenal gland was much lower than that of the gastric mucosa. These results suggest that PPI facilitates the estrogen synthesis in not the testis and adrenal gland but the parietal cells. The facilitation should cause an increment of the 17 β-estradiol level in the blood.

(COI: No)

P2-326

Analysis of the effect of retinoic acid on anterior pituitary cell functions in adult rat

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Retinoic acid (RA) is a lipid-soluble molecule, which is derived from vitamin A. This molecule serves as a ligand for two families of nuclear receptors that directly regulate gene expression, retinoic acid receptors (RARs) and retinoid X receptors (RXRs). RA is also one of the regulating molecules affecting pituitary cell functions. It activates growth hormone (GH) gene transcription. Moreover, RA stimulates the expression of D2R in lactotroph and suppresses TSH beta subunit expression in thyrotroph. However, the functions of RA on pituitary cells have not been well understood. The purpose of this study is to analyze the effect of RA on gene expression in primary pituitary cells in culture. The cells were isolated from anterior pituitary of adult Wistar rats. The cells were exposed to a graded concentration of all-trans RA (ATRA). In time-course experiments, the cells were cultured in the medium containing ATRA for 24 to 72 h. By using real-time PCR, we measured the expression level of GH releasing hormone receptor (GHRH-R) and GHS-R and SST-R in anterior pituitary cells. The treatment of ATRA (10^{-6} M, 24h) increased the expression of GHRHR and GHSR by 2 times. The stimulatory effect of ATRA on GHRH-R and GHS-R expressions were dose-dependent and time-dependent manner. These results suggest that RA controls the expression of receptors of stimulating factors and then induces the release of GH from somatotroph. In addition to GHRH-R and GHS-R gene expression, we have analyzed novel RA-induced or -suppressed genes in anterior pituitary cells by means of DNA microarray analysis.

(COI: No)

P2-327

Analysis of collagen producing cells in human anterior pituitary gland; normal pituitary and pituitary adenoma

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Extracellular matrix (ECM), especially collagen, is essential in the physiology of normal pituitary and in tumorigenesis, However the role of ECM and their changes in pituitary adenomas have not been well studied. To clarify this, we first identify the collagen producing cells in control tissue and pituitary adenomas by means of in situ hybridization and immunohistochemistry. Human pituitary adenomas with various type of clinical consistencies were obtained during surgery at Toranomon Hospital. We performed in situ hybridization for collagen I and III, and double stained by αSMA as a pericyte marker, and cytokeratin as an epithelial cells marker. In addition, we performed in situ hybridization for RGS5 which is also a pericyte marker. In pituitary adenomas, there are 4 types of collagen-producing cells, 1) myoepithelial like cell, 2) pericyte, 3) myofibroblast, 4) fibroblast. In hard elastic consistency typed tumors collagen producing cells are predominantly the myoepithelial like cell and myofibroblast. In soft consistency typed tumors, the majority of collagen producing cell is pericyte. We cathegorize and analyze the proportion of collagen producing cells type in each type of tumor. This study shows that there are alterations in collagen producing cells in normal pituitary and pituitary adenomas.

(COI: No)

P2-328

Expression of heparin-binding growth factor midkine/pleiotrophin family in the estrogen induced prolactinoma of rat

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Midkine (MK) and pleiotrophin (PTN) belong to a family of secreted heparin-binding growth factors. These growth factors have multiple functions, such as regulation of cell proliferation, migration, survival, differentiation, and tumorigenesis. We recently reported that MK and PTN mRNA is expressed in the anterior and posterior lobes of pituitary in adult rat. In the anterior pituitary gland, these mRNAs were expressed in the folliculostellate cells, which do not produce classical anterior pituitary hormones. It is suggested that MK and PTN play a role as paracrine signaling molecules in the pituitary gland. However, the presence of these growth factors in pituitary tumors has not been demonstrated. In this study, we examined the expression of MK and PTN in diethylstilbestrol-induced prolactinoma of LEXF RI rats. Adult male rats were subcutaneously implanted with a silastic tube containing diethylstilbestrol (DES) for 1 month, 2 months, and 3 months. Using in situ hybridization with digoxigenin-labeled cRNA probes, we detected cells expressing MK and PTN in prolactinomas. MK expression gradually decreased in the gland during tumorgenesis. On the other hand, PTN-expressing cells were increased in prolactinoma. Double-staining revealed that PTN mRNA was only expressed in folliculostellate cells in the adenoma. These results suggest that PTN relates to cell proliferation and tumorigensesis of lactotrophs.

(COI: No)

P2-329

Effect of human mesenchymal stem cells (hMSCs) administrated into pancreases on diabetic mice

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Diabetes is a chronic life-style disease which is characterized by hyperglycemia and destruction of insulin producing beta cells in the pancreatic islet. Although intravenous (iv) transplantation of human mesenchymal stem cells (hMSCs) temporary ameliorated the hyperglycemia in rodent model, stable and sustainable effect has not obtained yet. We examined here effect of local administration of hMSCs into pancreas (intrapancreas; ipan) on streptozotocin (STZ)-induced diabetic mice. C57/BL6 mice were injected STZ (115mg/kg, intraperitoneally) at day 0 and were transplanted hMSCs (10^6) either iv or ipan at day 7. Another set of the diabetic animals were transplanted hMSCs twice at 1 and 4 weeks, collected blood and pancreas at 8 weeks, and were measured insulin level in blood and pancreas. All animals were measured blood glucose during the experimental periods. STZ injection increased blood glucose within 1 week. hMSCs decreased the blood glucose levels both in iv and ipan groups, and ipan one was greater effect than iv one. Twice hMSCs injections lowered blood glucose to diabetic border line. The animals increased significantly body and pancreatic weight, and plasma insulin level. The animals also increased insulin-positive islet size, number and density compared with vehicle one. We demonstrated that ipan hMSCs were much effective strategy compared with iv one to ameliorate diabetic symptoms.

(COI: No)

P2-330

A new function of Neuromedin U/Neuromedin S system in the CNS

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Neuromedin U (NMU) is a neuropeptide which was isolated from porcine spinal cord in 1985. In 2000, its specific receptors, NMUR1 and NMUR2, were identified, then the physiological functions have been examined. We have reported that this NMU system has important roles in central regulation of food intake, energy expenditure, stress responses, and circadian rhythmicity by using gene genetically modified mice. Another endogenous ligand, Neuromedin S (NMS), was identified in 2005, and its physiological roles were reported as regulation of circadian rhythm and food intake. However, NMU/NMS system seems to have other unknown physiological functions. It was reported that NMUR2 was expressed in the nucleus accumbance (NAc) in the brain where is related with reward system (Brain Res Gene Expr Patterns, 2001, 1:1-4), and some other reports suggested that central Neuromedin U mRNA was up-regulated in addiction models related with reward systems (PLoS One, 2010, 5:e15643). Recently, the central regulation of food intake is suggested to be deeply related with "higher brain functions" including reward system, preference, and recognition. Based on these evidences, we are focusing on examining a higher brain function of NMU/NMS system related with energy homeostasis. Here, we have performed a series of behavioral test by using newly established NMU/NMS double knockout mice and found this double KO mice shows some abnormal behavior response, such as anti-anxiety behavior and will figure out the molecular mechanism of this new physiological functions of the central NMU/NMS system.

(COI: No)

P2-331

COA-Cl-induced adipogenesis is associated with an increased cell cycle progression and down-regulation of $p27^{Kip1}$ in mouse pre-adipocyte 3T3-L1 cells

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Mouse pre-adipocytes 3T3-L1 cells undergo mitotic clonal expansion upon induction of adipogenesis. This clonal expansion is associated with down-regulation of cell cycle inhibitors, $p27^{Kip1}$ and/or $p21^{Cip1}$. We found that COA-Cl, a synthesized adenosine analogue with pro-angiogenic activity, enhances lipid accumulation and adipocyte marker gene expression 8 days after treatment with 1 μg/mL insulin (Ins), 1 μmol/L dexamethasone (Dex), and 500 μmol/L 1-methyl 3-isobutylxanthine (IBMX) in 3T3-L1 cells. Here we examined the effect of COA-Cl on the cell cycle in 3T3-L1 cells after induction of adipogenesis. The cell cycle distribution was evaluated with a flow cytometric analysis of propidium iodide fluorescence. When 3T3-L1 cells were stimulated with Ins, Dex and IBMX in the presence of COA-Cl, the cell cycle distribution in G0/G1-phase decreased from 86 to 83% and that in S/G2-phase increased from 14 to 17% ($p<0.05$). The treatment with COA-Cl down-regulated the expression of $p27^{Kip1}$. These results suggest that COA-Cl-mediated enhancement of adipogenesis is associated with an increased cell-cycle progression and down-regulation of $p27^{Kip1}$.

(COI: No)

P2-332

Expression analyses of stress-related factors in single prolonged stress rats

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Post-traumatic stress disorder (PTSD) is a stress-related anxiety syndrome that develops after exposure to traumatic experience. Many individuals with PTSD remain chronically symptomatic, implying that sustained structural and functional changes in brain is predisposing factors of PTSD. However, until today, biological basis of PTSD is almost unknown. Single prolonged stress (SPS) is an established animal model proposed for PTSD, and mimic the phathophysiological and behavioral characteristics of PTSD. In this study, by using SPS paradigm, we investigated the expressions of corticotropin-releasing hormone (CRH) and glucocorticoid receptor (GR) in the brain of adult male rats. SD rats at 8 weeks, were subjected to a single session of prolonged stress consisting of immobilization, forced swimming, and exposure to ether vapor. Seven days after SPS treatment, mRNA and protein expression levels of CRH and GR were examined by real-time PCR and immunohistochemistry. In SPS rats, no change was detected in expression of GR protein in hippocampus. However, CRH mRNA and proteins showed significant increase in the central nucleus of the amygdala which is known to be involved in the expression of emotion such as anxiety and fear. These results suggest that SPS paradigm alters stress-related factors in mammalian brains, and may provide the physiological and behavioral basis of PTSD.

(COI: No)

P2-333

Effect of dominant negative thyroid hormone receptor in Purkinje cells to the cerebellar development

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Thyroid hormone (TH) deficiency during the fetal and early postnatal periods results in severe mental and physical retardation, known as cretinism in humans. We have generated a transgenic mouse line expressing a dominant-negative TH receptor (TR) in cerebellar Purkinje cells to study the role of the TH in cerebellar development. A mutant human TRβ1 (G345R) was subcloned into full-length L7/ Pcp-2 gene, which is specifically expressed in Purkinje and retinal rod bipolar cells. We confirmed the transgene localization specifically in Purkinje cells by immunohistochemistry. By Western blot, we detected the expression of the transgene from postnatal day (P)2. It was kept increasing during development. There is no significant retardation in general growth or cerebellar weight in Tg/Tg mice, which is compatible to no difference of plasma free T3 and free T4 levels. However, the motor coordination of Tg/Tg mice was significantly disrupted at P15 and P30 by Rotarod test. To elucidate the reason why cerebellar development was markedly delayed in the Tg/Tg mice, we examined the changes in the expression levels of TH-responsive genes using realtime quantitative RT-PCR. To our surprise, not only Purkinje cell-expressed genes, but also the other cell type-expressed genes were suppressed by mutant TRβ1 in Purkinje cells. These results indicate that TH action through TR in Purkinje cells is also important for development of other subsets of cerebellar cells such as granule cells.

(COI: No)

P2-334

Molecular mechanism of Neuromedin U system in NAFLD/NASH

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Neuromedin U (NmU), which is neuronal peptide isolated from porcine spinal cord, has multiple physiological functions including appetite suppression, stress response and so on. Some groups have reported the relationship between NmU and inflammation, however, its precise function has not been elucidated yet. NmU reacts with NmUR1 and NmUR2, known as specific receptors for NmU, and NmUR1 is mainly expressed in the peripheral tissues and NmUR2 in the central nervous systems. With that in mind, we are attempting to verify the participation of NmU system in obesity related inflammation disease such as nonalcoholic fatty liver disease/nonalcoholic steatohepatitis (NAFLD/NASH). First of all, we have examined NmU and its receptors expression levels in liver tissue of high fat diet (HFD) mouse model. Normally, it is not detected NmU mRNA in liver tissue without any pathological change, however, NmUR1 but not NmU expression level has some tendency to increase at HFD mouse liver. Next, we have examined diet induced NASH model and found that NmU and NmUR1 mRNAs are markedly increased in NASH liver. To assess what kind of cell type is expressed NmU in the liver tissue, we have performed to do immunohistochemistry and observed that NmU was co-expressed with macrophage specific marker, F4/80. More than this, NMU expression in NASH liver is detected not only in the mouse model but also in human NASH patient. These data suggests that NmU/NmUR1 pathway has some roles in NASH pathophysiology, and also NmU has a possibility to be a biomarker of NASH.

(COI: No)

P2-335

Trasncription factor MafA is critical for maintenance of the mature β-cell phenotype

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Pancreatic β-cells are highly differentiated and regulate blood glucose levels by secreting insulin in response to glucose stimulation. Insulin gene transcription factor MafA is expressed in mature β-cells, while MafB, another Maf family transcription factor, is expressed in immature β-cells. Pancreatic islets in MafA knockout (KO) mice were normal at birth, but lost insulin expression in β-cells with increased population of the glucagon-expressing α-cells over time. Analysis of mRNA expression in MafA KO islets at 7 weeks of age showed reduced expression of selective genes important for β-cell function. In parallel, the upregulation of genes that are normally "disallowed" in mature β-cells, such as Mct1, or transcription factors transiently expressed in endocrine progenitors was identified in MafA KO islets as a hallmark of dedifferentiation. By the lineage tracing analyses of β-cells in MafA KO mice using the Cre-LoxP system, the conversion of β-cells to the glucagon-expressing cells with reduced/lost expression of insulin was observed in MafA KO mice. TEM of MafA KO islets demonstrated numerous "empty" vesicles without insulin granules in β-cells. These results suggested that the maturation factor MafA is critical for the homeostasis of mature β-cells and regulates cell plasticity. The loss of MafA in β-cells leads to a deeper loss of cell identity, which is implicated in the pathology of diabetes.

(COI: No)

P2-336

Adrenaline modulates glucagon-like peptide-1 secretion from enteroendocrine L cells

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Adrenaline is known to induce diverse physiological responses in many tissues and cells through adrenergic receptors. Although enteroendocrine L cells secrete glucagon-like peptide-1 (GLP-1) in response to the variety of nutrients, neurotransmitters, and hormones including adrenaline, the precise molecular mechanism by which adrenaline-induced GLP-1 secretion have been poorly understood. To clarify the molecular mechanism of adrenaline-induced GLP-1 secretion, we used enteroendocrine L cell line, GLUTag cells. RT-PCR analysis showed that all types (α_1, α_2, and β) of adrenergic receptors were expressed in the cells. Application of adrenaline to the cells induced a marked increase of the intracellular calcium concentration ($[Ca^{2+}]_i$) and GLP-1 secretion. Inhibition of Gq signaling pathway significantly inhibited the increase of $[Ca^{2+}]_i$ and the adrenaline-induced GLP-1 secretion. Although the intracellular cAMP concentration ($[cAMP]_i$) was little changed by the application of adrenaline, overexpression of α_2 adrenergic receptors induced the decrease of $[cAMP]_i$ by application of adrenaline. These results suggest that expression spectrum of α_1, α_2, and β adrenergic receptors in the cells would modulate the amount of GLP-1 release.

(COI: No)

P2-337

Comprehensive expression pattern analysis of a tumor suppressor gene, *REIC/Dkk3* in the mouse

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In recent tumor suppressor and therapeutic gene research, the reduced expression in immortalized cells (REIC)/Dickkopf (Dkk)3 gene has attracted many researchers. Previous studies demonstrated that the intratumoral introduction of *REIC/Dkk3* gene suppresses tumor growth in mouse models of prostate, breast and testicular cancer and malignant mesothelioma, suggesting that *REIC/Dkk3* is a tumor suppresser gene. However, the functions of *REIC/Dkk3* in vivo are widely unknown. Here, we investigated the comprehensive expression pattern of *REIC/Dkk3* in adult mice by in situ hybridization and immunofluorescence analyses. Firstly we made frozen sections of major mouse organs and performed in situ hybridization. *REIC/Dkk3* mRNA was strongly expressed in many organs, such as the brain, eye, heart, thymus, adrenal gland, testis, ovary and gastrointestinal tracts. In contrast, *REIC/Dkk3* was weakly expressed in the spleen and pancreas. We further performed immunofluorescence analysis for REIC/Dkk3 protein on the tissues in which intense *REIC/Dkk3* mRNA expression was observed. We found that REIC/Dkk3 protein was specifically present in certain cells of these organs. Unexpectedly, REIC/Dkk3 protein was detected intensely in the lumen of digestive organs. In summary, the central nervous system, digestive system and genital organs had strong *REIC/Dkk3* expression. In addition, REIC/Dkk3 protein appeared to be secreted to the lumen of digestive organs.

(COI: No)

P2-338

Investigation of FABP7 expression in mouse Kupffer cells

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Fatty acid binding proteins (FABPs) are intracellular carriers of long chain fatty acids. They have 12 different subtypes with spatial and temporal differences in their tissue expression patterns. We previously reported that FABP7 is involved in cytokine production upon LPS stimulation and phagocytosis against apoptotic cells in liver Kupffer cells (KCs). However, the mechanism by which FABP7 expression is regulated in the liver environment is still unknown. In this study, we first examined the detailed localization of FABP7 by immunohistochemistry in the mouse liver during early postnatal period. Next, in order to see how bone-marrow derived cells acquired FABP7 expression, we examined the occurrence of FABP7+ macrophages in the liver using mouse models treated with clodronate-liposome and of bone marrow transplant. In immunohistochemistry, KCs started to show FABP7 expression from postnatal day 4 (P4), while F4/80+ cells were constantly detected in the liver from P0 to P10. Although FABP7 was not detected in the blood monocytes, it was expressed in the newly derived macrophages in the liver after KC-depletion by clodronate-liposome treatment or in the bone marrow transplant model. The results strongly indicate that the environment provided by a mature liver are required for FABP7 expression in KCs.

(COI: No)

P2-339

The three-dimensional reconstruction of serial sections for an analysis of the microvasculature of the human spleen

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The spleen acts as a blood filter and is responsible for the immune response against blood-borne antigens. Although a number of papers have been published on the splenic microcirculation by light and/or scanning electron microscopy, some controversies still remain on the vascular arrangement, especially in the human spleen. Thus, the aim of the present is to reexamine the microvasculature of the human spleen by using a three-dimensional (3D) reconstruction technique of immunohistochemically stained tissue sections. Human spleens (obtained from patients with gastric cancer) were perfused with warmed physiological saline via the splenic artery, followed by fixation with 4% paraformaldehyde. Serial sections were made from the paraffin or Epon-embedded tissue blocks, immunostained for CD34 (a marker for blood vessel), and observed by light microscopy. The 3D reconstruction was made from the serial section images using a computer program (Avizo, VSG inc., France). Our findings revealed that the splenic follicle is surrounded by anastomosed capillaries, which is elaborately developed in the marginal zone. Most of these capillaries are branches of the penicilar arterioles coming apart from the central artery in the different white pulp system. Further details will be demonstrated especially on the spatial relationship between blood vessels and parenchymal tissues.

(COI: No)

P2-340

Diversity of the peritoneal mesothelial cells: Germinal epithelial cells of the ovary in mice

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Benign lymphangiomas can be easily induced by Freund's incomplete adjuvant (FIA), but the tumors reveal some diversity in their components. Our previous studies demonstrated that a single intraperitoneal injection of FIA into young adult mice induced the phenotypic transformation of peritoneal mesothelial cells to form lymphangiomas. They become tall as early as 5-7 days after the injection and lost their polarity by detaching each other within 4 weeks after injection. The tumor cells increased and uptook a lot of FIA as cytoplasmic vacuoles of various sizes, resulting in typical lymphangiomas with honey-comb like features. As they grew, the positivity of podoplanin (Pdp), one of lymphatic endothelial markers, became much more evident in lymphangioma cells than in the normal mesothelial cells. On the other hand, surface (or germinal) epithelial cells in the ovary did not form any tumor mass, although they become strongly positive for Pdp similarly to other peritoneal mesothelial cells forming lymphangiomas at different sites in the peritoneal cavity. These results suggest that the peritoneal mesothelial cells may have a diverse potentiality for their phenotypic transformation depending on the site of peritoneal cavity.

(COI: No)

P2-341

Transforming growth factor beta 2 from folliculostellate cells induces collagen synthesis in pericytes of rat anterior pituitary gland

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Anterior pituitary gland consists of endocrine cells and non-endocrine cells. These cells are distributed in an appropriate manner within lobules, which are surrounded by extracellular matrix such as collagens. It is considered that the structural properties are important in the maintenance of anterior pituitary functions. Our recent study showed that folliculostellate (FS) cells are required for collagen synthesis of pericytes. However, a factor that induces collagen synthesis in pericytes has not been identified. In the present study, we investigated whether FS cells express a major fibrogenic cytokines, transforming growth factor beta family (TGFb1-3). We performed RT-PCR, in situ hybridization, and immunohistochemistry to detect TGFb and their receptors (TGFBRI-III), and utilized primary cell culture of rat anterior pituitary to examine the effects of TGFb on collagen synthesis. RT-PCR and in situ hybridization revealed that TGFb2 was expressed in the FS cells and the TGFBR-II was expressed in the pericytes. TGFb2 induced Smad2 nuclear translocation in the pericytes and increased collagen synthesis dose dependently. These results suggested that TGFb2 secreted from FS cells affects on pericytes to induce collagen synthesis. Currently, we are investigating the effects of TGFBR inhibitor on collagen synthesis to confirm the action of endogenous TGFb2 actions.

(COI: No)

P2-342

KGF/KGFR control on the epithelial cell proliferation of mouse ear skin

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Keratinocyte growth factor (KGF) is a mesenchymal-cell-derived paracrine growth factor that specifically stimulates epithelial cell growth. In this study, we investigated the effects of over-expressed KGF during epithelial cell proliferation by using a cell labeling system. After anesthetized ICR mice Flag-hKGF cDNA (provided by Dr Jeffrey S. Rubin, the National Cancer Institute/CCR/LCMB) was transfected into ear skin with electroporation. KGFR selective inhibitor (SU5402) was administered in some ears after vector transfection. At 1, 4 and 7 days after transfection, 9 mice at each time-point were sacrificed. For direct assessment of DNA synthesis activity, 5-bromo-2'-deoxyuridine (BrdU) was injected 24 h prior to vector transfection and 5-ethynyl-2'-deoxyuridine (EdU) was injected 2 h prior to each sacrifice. Immunohistochemistry for Flag, KGF, KGFR, BrdU and keratin (K)14 was performed. EdU was detected by manufacturer's protocol. Each plasmid was transfected into the epithelial and subepithelial cells, successfully. After KGF transfection, keratin accumulations were observed at DAY 4. Moreover, increased number of BrdU(-)EdU(+) cells were detected at DAY 1 and BrdU(+)EdU(+) cells were detected in the upper layer of thickened K14 positive epithelium at DAY 4 after KGF transfection. The treatment with SU5402 prevented BrdU(-)EdU(+) or BrdU(+)EdU(+) cell proliferation completely. These findings indicated that KGF may possibly induce a progenitor cell proliferation of epithelium and KGFR inhibitor may be a possible drug for proliferative dermatitis.

(COI: No)

P2-343

Ontogeny of localization of epithelial sodium channel (ENaC) in bullfrog ectoderm

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An ENaC is an ion channel (consisting of three subunits: α-, β-, and γ-ENaC) that is concerned with Na^+ absorption across epithelia. Adult bullfrog skin is an osmoregulatory organ and it absorbs Na^+ ions from its apical to basolateral side via ENaC. Application of amiloride (Am), a specific inhibitor of the α-ENaC subunit, to the adult skin's apical side inhibits such absorption. Amphibia change habitat from aquatic to terrestrial during their developmental stages. The functions of the skin, such as Na^+ absorption, are directly affected by environmental conditions, and the role of the α-ENaC in such functions must change during development. We generated an anti-α-fENaC (anti-α-ENaC against bullfrog) and by immunohistochemistry used it to investigate when α-ENaC develops in bullfrog skin and the related organs. An α-fENaC signal was not detected in stage (St.) 13 embryos, but α-fENaC was expressed in cement glands in Sts. 18, 19, and 21 embryos. The signal was also detected in gills in Sts. 21 and 23-24 embryos. In the skin, α-fENaC was expressed in St. 21 embryos and thereafter (i.e., in larval skin and adult skin), but its activity (Am-blockable response) was not detected in larval skin. The reason for this discrepancy was that α-fENaC was not localized in the apical plasma membrane of such larval skin.

(COI: No)

P2-344

Live-imaging of the basement membranes in mammalian systems

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Basement membranes (BMs) are thin sheet-like extracellular matrix found beneath epithelial cell layers of multicellular organisms. The epithelial cells adhere to and receive signals from BMs through cell surface receptors. Vice versa, the formation of BMs are tightly regulated by neighboring cells. The cell-BM interactions play significant roles in many biological processes including epithelial morphogenesis during embryonic development. Several studies using hydra, worm, and insects demonstrated that the BMs are dynamic structures. However, the dynamics of BMs in mammalian development are largely unknown. Here we developed a novel probe to visualize the BM dynamics in mammalian systems. Nidogen-1, a ubiquitous BM protein was applied to label BMs. Recombinant human Nidogen-1 fused with green fluorescent protein (nid1-EGFP) was added to the culture medium of embryoid bodies (EBs), a 3D model of BM formation, derived from mouse embryonic stem cells. The nid1-EGFP was successfully incorporated into the BMs formed in EBs. Continuous observation revealed that the incorporation of nid1-EGFP was observed not only at the beginning of the EB-differentiation but also in the later stages, suggesting that the BMs in EBs were formed and maintained under continuous turnover. The probe we developed for live-imaging of BMs will provide a powerful tool to study the mechanisms of morphogenesis.

(COI: No)

P2-345

Immunohistochemistry of adult rat dorsal root ganglion neurons

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Adult rodent dorsal root ganglion (DRG) neurons can be divided into three principal subgroups by their soma size and characteristic markers; large neurons (immunoreactive for 200 kDa neurofilaments (NF200), small peptidergic neurons (immunoreactive for calcitonin gene-related peptide (CGRP) and high-affinity nerve growth factor (NGF) receptor trkA), and small non-peptidergic neurons (immunoreactive for glial cell line-derived neurotrophic factor (GDNF) family receptor components (RET, GFRα1) and binding to isolectin B4 (IB4)). By employing double immunofluorescent staining with the markers for the respective subgroups (i.e. anti-NF200 and anti-CGRP antibodies, and Alexa Fluor dye-conjugated IB4), we investigated the precise localization of galectin-1 (GAL-1), galectin-3 (GAL-3), and glucagon-like peptide 1 receptor (GLP-1R) in the sections of 3-month-old Wistar rat DRG. Both GAL-1 and GAL-3 were predominantly localized at IB4-binding small non-peptidergic neurons, whereas GLP-1R was predominantly localized at NF200-immunoreactive large neurons and CGRP-immunoreactive small peptidergic neurons. Consistent with such distribution patterns, GAL-1 and GAL-3 are involved in the neurotrophic activities of GDNF on cultured DRG neurons, and exendin-4, a GLP-1R agonist, restores pyridoxine-induced large fiber neuropathy and diabetes-induced large and small fiber dysfunction. These findings illustrate the correlation of immunohistochemical localization of the individual molecule with its functional roles in the sensory nervous system.

(COI: No)

P2-346

Characterization of the GFAP-like gene which is expressed in the brain of the Japanese newt *Cynops pyrrhogaster*

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In our study we have observed the Japanese newt medulla oblongata by immunohistochemistry with the anti-glial fibrillary acidic protein (GFAP) antibody. GFAP is an intermediate filament specific to astrocytes. Ependymal cells lining the fourth cerebral ventricle showed GFAP immunoreactivity, and their processes which ran through the gray and white matter also showed positive reactivity. The white matter contained a few neuroglial cells and a few positive cells. To clarify the expression of the GFAP gene in the newt's brain, we attempted to isolate the GFAP cDNA clones by RT-PCR method. Total RNA was isolated from brain tissues of the adult *Cynops pyrrhogaster* under anesthesia. The primers were designed based on the known GFAP sequence of vertebrates. We used 5' RACE method to obtain the 5' end of the provided cDNA by RT-PCR. As a result, about a 1kbp partial sequence was obtained. This sequence showed partial similarity with the GFAP of other vertebrates. Because the provided sequence could be read in a continuous amino acid sequence, it has the potential to be in the inside of an ORF. The amino acid had a sequence similar to GFAP, lamin and neurofilament. We made artificial polypeptides from two regions of unique amino acid sequences. Polyclonal antibodies were raised against these polypeptides. Proteins extracted from the newt's brain were separated by SDS-PAGE and analyzed by the immunoblotting procedure. We recognized a single-labeled band of about 60kD. This data suggest that we have cloned the partial cDNA sequence which encodes one of the intermediate filaments.

(COI: No)

P2-347

Localization of ionotropic glutamate receptor mRNAs in the pigeon retina

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Organization of the retina conserves well in the vertebrate. In mammals, main cells in the retina consist of glutamatergic neurons (photoreceptor cell, bipolar cell, ganglion cell) and GABAergic neurons (horizontal cell and amacrine cell). In birds, it is known that bipolar cells and ganglion cells are glutamatergic and amacrine cells are GABAergic. However, cell types receiving glutamate from glutamatergic cells remains unknown well. In the present study, distribution of mRNAs for ionotropic glutamate receptor subunits was examined to detect receptor cells in the pigeon retina by in situ hybridization. Adult pigeons were perfused with 4% paraformaldehyde under anesthesia and frozen sections of eye balls were cut on a cryostat. Several gene probes for in situ hybridization were used: glutamate receptors for AMPA (GluA1, 2, 3, 4), kainate (GluK1, 2, 4) and NMDA (GluN1, N2A) types, vesicular glutamate transporter 2 (vGluT2) and glutamic acid decarboxylase 65 (GAD65). VGluT2 mRNA was expressed in bipolar cells, amacrine cells, and ganglion cells. GluA1, 2, 3, 4 were expressed in bipolar cells, amacrine cells and/or ganglion cells. GluK1, 2, 4 were localized in bipolar cells and ganglion cells. GluN1 and N2A were expressed in an inner part of the inner nuclear layer and ganglion cells. GAD65 was strongly expressed in an inner part of the inner nuclear layer. The present study showed that bipolar cells and ganglion cells are glutamatergic and they express several ionotropic glutamate receptors in the pigeon retina.

(COI: No)

P2-348

Melanin pigmentation and Pacinian corpuscles in human juxta-oral organ

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The juxta-oral organ (JOO), which is first reported in 1885 by the Danish anatomist J. H. Chievitz, exists bilaterally in the bucca of all mammals and some reptiles. It consists of a central epithelial cord, embedded in connective tissue rich in nerve fibers and sensory receptors. In spite of over 100 years of studies, little is known about its precise structure and function, probably because of the difficulty of macroscopical dissection of human JOO. According to Zenker (1982), human JOO is 7-17mm in length and 1-2mm in diameter, and is possible to be dissected under a surgical microscope. However, there have been no reports that Japanese cadaveric JOO was macroscopically dissected, but only two autopsy case reports in which existence of melanin pigmentation and Pacinian corpuscles were pointed out. But their incidences are unknown. In this study, existence and incidences of melanin pigmentation and Pacinian corpuscles were studied by analyzing cadaveric JOOs. Serial sections of eighteen blocks of buccal tissues obtained from fourteen Japanese cadavers were made and HE staining was performed on them. After confirming the location of JOO, existence of melanin pigmentation and Pacinian corpuscles in the organ were observed. Among eighteen specimens, JOO was confirmed in fifteen cases, in which melanin pigmentation was observed in ten cases and Pacinian corpuscles in none. These results suggest that the existence of melanin pigmentation is common while the existence of Pacinian corpuscles is rare case in Japanese JOO.

(COI: No)

P2-349

Age- and sex-dependent changes in prosaposin and its receptors in the lacrimal glands of rats

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Saposin-deficient patients have various ophthalmic disorders indicating a relationship between ocular diseases and prosaposin (PS). Although the lacrimal glands are responsible for maintaining normal ocular health, and dysfunction of these glands causes many ophthalmic disorders, there is a paucity of information regarding PS and the lacrimal glands. In this study, we investigated the changes in PS and its receptors, GPR37 and GPR37L1, in the lacrimal and extraorbital lacrimal glands of rats using immunohistochemistry. We used young (1 month old), growing (2 months old), and adult (18 months old) male and female rats. A histological analysis revealed that PS immunoreactivity in the lacrimal glands decreased with age in male rats, while the opposite result was observed in female rats. On the other hand, a higher level of PS was observed in the extraorbital lacrimal glands of young and adult rats of both sexes. GPR37L1 was more highly expressed than GPR37, and showed decreasing trend with age in both glands of male rats while very low levels were detected in female rats. In fact, the female rats showed a lower level of PS and its receptors than male rats at all ages examined. In conclusion, we found age and sex differences in the levels of PS and its receptors in rat lacrimal gland, suggesting different roles for PS in the maintenance of ocular health in male and female rats at different ages.

(COI: No)

P2-350

Alternative reaction of Harderian gland after lacrimal gland removal in mice

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Harderian gland is an exocrine gland present in most groups of vertebrates. Harderian gland locates within the eye orbit, and secretes lipids (oil fluid) on surface of eyes for protection of eye. We recently established mouse dry eye model by removal the lacrimal glands. The dry-eye mouse caused corneal damage at 1 week after removal operation, but the corneal damage was gradually recovered and looks normal at 8 weeks. We check the tear volume level, but it keeps less than 20% before operation for 8 weeks. Interestingly, weight of the harderian glands significantly increased from 2 weeks after lacrimal gland removal. The number of Ki67, proliferation marker, immunopositive cells in harderian gland significantly increased and peaked at 1 week after lacrimal gland operation. Then we checked the lipid metabolism genes mRNA levels by real-time PCR analysis. In the results, lipoprotein lipase, which relating promotion of the cellular uptake of lipoproteins and free fatty acids, level significantly increased at 3 days. Conversely, monoacylglycerol lipase, relating hydrolysis of monoglycerides to lipoprotein triglycerides, level significantly decreased at 1 and 2 weeks after removal operation. These results suggesting that harderian gland alternatively caused hypertrophy and hyperfunction for protecting the cornea after lacrimal gland removal.

(COI: No)

P2-351

α1-Adrenoceptors relate Ca^{2+} modulation and mucin secretion in rat lacrimal grand

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The lacrimal gland is responsible for secretion of electrolytes, water and proteins, collectively known as lacrimal gland fluid, into the tear film. Cellular secretory activities are enhanced by noradrenaline (NA) as well as by cholinergic stimuli. Here, lacrimal gland acinar cells response to adrenoceptors activation were examined, with special reference to intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) dynamics. Detection of mRNA of acinar cells specific to adrenoceptor subtypes was determined by RT-PCR. All kinds of adrenoceptors were detected except α2c and β1 in lacrimal glands. NA induced an increase in $[Ca^{2+}]_i$ in acinar cells, and these $[Ca^{2+}]_i$ changes showed a biphasic behavior. The removal of extracellular Ca^{2+} and the use of Ca^{2+} channel blockers did not inhibit the NA-induced $[Ca^{2+}]_i$ increases. In addition U73122 did not completely block the NA-induced $[Ca^{2+}]_i$ increase. Phenylephrine induced a atrong increase in $[Ca^{2+}]_i$. However, clonidine and isoproterenol failed to induce a [Ca2+]i increase. Mucin secretion was quantified by the peroxidase activity in rat lacrimal glands. Ca^{2+}-dependent exocytotic secretion of peroxidase was detected in rat lacrimal glands. RT-PCR results showed that Muc1, Muc5AC, Muc5B, and Muc16 mRNA were expressed in acinar cells. These findings indicated that NA activates α1 adrenoceptors which cause an increase in $[Ca^{2+}]_i$ by production of IP_3 and these receptors were main receptors in calcium-related cell homeostasis and protein (mucin) secretions in lacrimal glands.

(COI: No)

P2-352

The distribution of PACAP receptor on the sweat glands

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Pituitary adenylate-cyclase activating polypeptide (PACAP) has pleiotropic functions that contribute to neurotransmission, neuroprotection and vasodilatation. In addition, PACAP has been shown to influence the activity of several exocrine glands. Recently, we reported that PACAP and its receptor, PAC1R are localized to the parasympathetic nerve cells in the mouse lacrimal gland. Furthermore, PACAP instillation induced tear secretion. As structure of sweat glands and lacrimal glands structure are similar, we assumed that PACAP has a capacity to induce sweating in the sweat glands. However, its effects on the composition of the sweat glands are not known yet. To investigate the localization of PACAP and PAC1R in mouse sweat glands, we performed immunohistochemistry and RT-PCR. In this study, we examined PACAP, PAC1R, VPAC1, VPAC2 and VIP expression in mouse footpad by RT-PCR. The expressions of all these genes were detected in mouse footpad. In addition, we demonstrated that Cytokeratin (CK)19 was expressed in both ductal and secretory cells of sweat glands in mouse foot pad. By immunohistochemical staining, we found that PAC1R was expressed in the mice footpad consistent with the position of the sweat glands which were identified by CK19. Abundant PAC1R immunoreactivity was observed in secretory cells and in the excretory duct of sweat glands. The present results suggest that PACAP has an important role in secreting sweat in the sweat glands. For farther study, we are now examining the sweat secretion after PACAP administration in vivo.

(COI: No)

P2-353

Possible participation of synovial lining cells in vascularization in the rat temporomandibular joint

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The lining layer of the synovial membrane in the temporomandibular joint (TMJ) contains two types of synovial lining cells (SLC): a macrophage-like type A cell and fibroblast-like type B cell. We previously demonstrated that the desmin-positive B cell resembles an activated pericyte during angiogenesis in both immunoreactivities and ultrastructure in rat TMJ. The present study investigated the immunocytochemical characteristics of the SLC, focusing on their possible participation in the synovial vasculature. Adult male Wistar rats were used. Some animals were intravenously injected with FITC-labeled tomato lectin before fixation. Decalcified cryostat sections were processed for immunohistochemistry using antibodies to desmin, RECA-1 (endothelial marker), and ninein (sprouting tip cell marker). Ultrastructurally, RECA-1-positive SLC--not luminal in shape--were type A cells. Lectin-perfusion enabled the representation of functional vessels in vivo in the TMJ. Arterioles and venules in the synovial folds with RECA-1-positive endothelial cells and desmin-positive pericytes gave rise to numerous capillaries which were distributed densely in the synovial lining layer. Some capillaries with RECA-1-reaction lacked lectin-staining in the distal portion, indicating a loss of blood-circulation due to vessel sprouting or obliteration. RECA-1 and desmin-positive SLC often attached to this portion. A few capillaries also expressed ninein. These findings suggest that SLC might contribute to angiogenesis in the synovial membrane.

(COI: No)

P2-354

Lymphangiogenesis and NOS localization in the healing process after tooth extraction in a mouse diabetes model

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Objective: Type I diabetes causes the dysfunction of vascular endothelial cells comprising blood and lymph vessels, and arteriosclerosis progresses due to reduced nitric oxide (NO) production, showing a close association between NO and diabetes. In this study, we immunohistochemically investigated lymphangiogenesis and NOS expression in the healing process after tooth extraction using the Akita mouse diabetes model.
Materials and Methods: The lower first molar was extracted in C57L/6J and Akita mice at 12 weeks after birth. The mandible was excised and decalcified with 10% EDTA. After embedding in paraffin, immunohistochemical staining with antibodies against nNOS, iNOS, eNOS, VEGF-C, VEGFR-3、and vWF was performed employing the standard procedure, and the preparations were observed under a light microscope.
Results: Among the time-points: 1, 4, and 10 days after tooth extraction, the strongest reactions of NOS, VEGF-C and R-3, and vWF were noted at 4 days. At 10 days, osteoblasts in formed bone were positive.
Discussion: It was suggested that the host defense mechanism and vasodilatory action became active 4 days after tooth extraction, leading to lymphangiogenesis. Osteoblasts were activated at 10 days, suggesting active osteogenesis in Akita mice.

(COI: No)

P2-355

A histopathological study of possible visceral mycosis in Medaka(*Oryzias latipes*)

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Background: Medaka (*Oryzias latipes*) is widely used as a vertebrate model animal in various research fields. We have used medaka populations as an analogy of human populations, in order to understand the relationship between genetic polymorphisms and phenotypes, including genetic and or inflectional disease. The problem is however, there are little pathological data. To provide substantial information on histological and pathological aspects of medaka, we have prepared the whole-body serial tissue sections of the medaka with aberrant formation, and performed histopathological observations.
Materials and Methods: An individual medaka in a laboratory closed colony derived from a wild population caught in Kunming, China, showed serious abdominal swelling and examined. It was fixed for 10 days in Davidson solution, and then the conventional method was used for preparing the whole-body serial tissue paraffin sections at intervals of 5 μm. After the deparaffinization, the sections stained by HE and PAS and Grocott were investigated under an optical microscope.
Results and Discussion: Through three types of staining, we observed visceral mycosis like- diagnoses were widely observed in the organs including kidney and ovary. Since such an infection of fungus in organs in medaka has not been reported so far, it must be valuable finding if the diagnoses are truly visceral mycosis.

(COI: No)

P2-356

Effect of quadriceps femoris muscle contraction by electrical stimulation before bicycle ergometer exercise on physical energy metabolism

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This study was performed to clarify the effect of quadriceps femoris muscle contraction (MC) by low-intensity transcutaneous electrical nerve stimulation (TENS) before bicycle ergometer exercise on physical energy metabolism. All healthy male volunteers (n = 6) were participated two experiment groups. In MC group, quadriceps femoris muscles of subject were passively contracted by low-intensity TENS for 30 min before bicycle ergometer exercise. In rest group, subject was rested on the bed for 30 min before the bicycle ergometer exercise. In both groups of MC and rest, the subject performed ramp incremental exercise to exhaustion on bicycle ergometer. Plasma free fatty acid (FFA) concentration before bicycle ergometer exercise in MC group has tended to be higher than that in rest group, but there was no significant difference between the two groups. The ventilation threshold (VT) shown by the exercise load during bicycle ergometer exercise in MC group was significantly higher than that in rest group ($p < 0.05$). There was no significant difference of blood lactate concentrations after bicycle ergometer exercise between the two groups. These results give a hypothesis that the localized muscle contraction by TENS before bicycle ergometer exercise might increase recruitment of FFA from adipose tissue, and might change the proportion of the substrate of ATP synthesis during bicycle ergometer exercise.

(COI: No)

P2-357

A possibility to promote the recovery from fatigue after submaximal pedaling exercise by bathing with high concentration CO_2-water in healthy subjecs

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We investigated an influence of immersion into bathtub water containing comparable amount of CO_2 to CO_2-hot spring to recovery from fatigue caused by submaximal exercise. Six male subjects (Age; 21-22 yrs) performed 10 min pedaling exercise at 60% HRmax were given one of the following 3 treatments after the exercise in a different day; immersion into tap-water (CO_2p<20 ppm) or artificial CO_2-water (CO_2>1000 ppm) (30℃, 10 min), or dry bathtub sitting rest (Air). Blood flow in the immersed skin (BF) and ECG were recorded continuously throughout the experiment. Cardiac autonomic nerve activity was evaluated by R-R interval fluctuation power spectrum analysis (PSA). Muscles stiffness (MS), salivary cortisol (SCo), VAS were evaluated at pre-exercise, immediately after exercise, during immersion and at 10 min after the end of immersion. At 10 min after immersion, MS in CO_2-water treatment was significantly small (22.2± 1.2 tone, $p<0.01$) compared with air (28.0± 2.0 tone) and tap-water treatment (31.8± 2.2tone). A LF/HF ratio in PSA was smaller in CO_2-water treatment than in tap-water treatment. Compared with the air, SCo was significantly decreased in tap (24%) and CO_2-water immersion (48%). Results suggested that the recovery from enhanced sympathetic nerve activity and MS caused by submaximal exercise was facilitated by following CO_2-rich water immersion.

(COI: No)

P2-358

Inhibition of fibrinolytic activity in overweight young men after acute strenuous exercise

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Introduction: Some studies have reported an increase in fibrinolytic activity after acute strenuous exercise. Conversely, fibrinolytic activity is inhibited in overweight person. In this study, our aim was to evaluate whether being overweight affects fibrinolytic activity after acute strenuous exercise. Being overweight was defined using body mass index (BMI) as a measure of the degree of obesity.
Subjects and Methods: Twelve healthy young men aged 19 to 23 years old who engaged in daily exercise participated in this study. Seven of these men were categorized in the BMI < 25 group, and five were in the BMI > 25 group. Venous blood samples were collected from the subjects pre- and post-performance of the Cooper test. This test involved running as far as possible within a 12-minute period. α 2-plasmin inhibitor / plasmin complex (PIC, as a marker of fibrinolytic activity) levels were measured using the collected blood samples.
Results: The PIC levels increased significantly in the BMI < 25 group (pre: 0.5 ± 0.02 μg/mL, post: 1.9 ± 0.3 μg/mL, $p < 0.05$), but these were not significantly increased in the BMI > 25 group (pre: 0.5 ± 0.08 μg/mL, post: 1.0 ± 0.1 μg/mL, $p > 0.05$).
Conclusions: Using BMI as an index for evaluation, this study showed that fibrinolytic activity is inhibited in overweight young men after acute strenuous exercise.

(COI: No)

P2-359

An influence of local bathing on recovery of performance after muscle fatigue: a pilot study comparing the effect of CO_2 enriched- and normal-tap water

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Soaking the body into CO_2 hot spring evokes prominent vascular dilation in skin and skeletal muscle of underwater portion of the body, and the skin reddens within several minutes, even in relatively low water temperature. Hence, we hypothesized that muscular fatigue may recover effectively due to facilitation of the local blood flow. To investigate the hypothesis, 9 healthy subjects (5 male, 4 female) immersed forearms into water (35 ℃) for 5min resting between sets. A set consists of a series of continual 30-times grip measurements for producing muscle fatigue. The grip measurements were carried out in both hands simultaneously. Forearms treatments during resting between sets were as follows: 1) without immersion, 2) immersion of one side into CO_2-water (CO_2>1000ppm) and another side into general tap water. Because sexual difference in muscle fatigue was not observed so far, results were analyzed without distinguishing the sex. The grip decreased by the 30th measurement by approximately 40% in each set, but a significant difference caused by treatment of the forearm during resting between sets was not observed, changes in muscle blood flow as well. The first grip of each set was significantly decreased as a set advances. Though a significant difference between forearm treatments with two kinds water was not detected under the present experimental conditions, recovery of muscle performance from fatigue was significantly promoted by water immersion after the exercise.

(COI: No)

P2-360

Eccentric muscle contraction stimulates mTOR signaling in human skeletal muscle

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The mammalian target of rapamycin (mTOR) signaling pathway has a key role in stimulating muscle protein synthesis and muscle hypertrophy. An acute bout of resistance exercise is well known to increase mTOR signaling in human skeletal muscle. However, whether concentric or eccentric muscle contraction stimulates mTOR signaling in human skeletal muscle remains unclear. The purpose of the present study was to investigate the effect of concentric and eccentric muscle contraction on mTOR signaling in human skeletal muscle. Sixteen young males performed 10 unilateral isokinetic concentric or eccentric knee extensions (90 deg/s) × 4 sets. Muscle biopsies (~15 mg) were obtained from the vastus lateralis 40 min before and 1 h after the resistance exercise and analyzed using the immunoblotting. The average peak torque for each of the four sets of 10 maximal eccentric contractions was significantly higher than that in concentric contractions. The blood lactate concentration immediately after eccentric contractions was significantly lower than that in concentric contractions. Eccentric muscle contractions significantly increased mTOR(Ser2448), S6K1 (Thr421/424), S6(Ser235/236), eIF4E(Ser209), p38(Thr180/Tyr182) and ERK1/2(Thr202/Tyr204) phosphorylation compared with those of concentric contractions. These results suggest that eccentric muscle contraction increases mTOR signaling in human skeletal muscle, which may be leading to muscle hypertrophy.

(COI: No)

P2-361

The forced respiration during the deeper upright water immersion causes the greater inspiratory muscle fatigue in healthy young men

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The purpose of this study was to evaluate the influence of the inspiratory load breathing (ILB) with various depths of water immersion on inspiratory muscle fatigue.
Methods: Eight healthy men (21.3 ± 0.5(SD) years) participated in three trials in random order, i.e., the subjects performed ILB during upright water immersion up to the umbilicus, 4th-rib, or clavicles. Maximum inspiratory pressure (PImax) was assessed in a sitting position with water immersion up to umbilical level before (baseline, BL) and immediately after the ILB. The 15-min ILB was performed with a respiratory frequency of 15 breaths/min and with a load of 30% PImax at BL. The percent changes in PImax following the ILB (δ %PImax) were calculated.
Results: The PImaxs were significantly decreased following the ILB in all trials ($p<0.05$). The δ %PImax was significantly greater in the clavicle-trial than those in the other trials (umbilicus, -7.0 ± 1.5(SD) %; 4th-rib, -6.7 ± 4.8%; clavicle, -20.1 ± 4.1%; $p<0.05$).
Conclusion: Our results suggested that the forced respiration during the upright water immersion up to the clavicular level resulted in the greater inspiratory muscle fatigue than those in the shallower levels, probably due to the increased demand on the intense inspiratory muscle strength to expand the chest against the greater hydrostatic pressure.
(COI: No)

P2-362

The effect of postural change on the distribution of cerebral blood flow during passive heating in elderly

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PORPOSE: The distribution of blood flow to cerebral tissues may be limited by heat stress and further by postural change from supine to upright. The purpose of this study was to assess the responses in the internal carotid artery (ICA), external carotid artery (ECA) and vertebral artery (VA) to postural changes during normothermia (NT) and mild-hyperthermia (HT).
METHODS: Ten elderly healthy men (72 ± 2.1 yrs, mean ± SD) underwent measurement of blood flow in the common carotid artery (CCA), ICA, ECA and VA by ultrasonography in a sitting (SIT) and supine (SUP) during NT and HT. Subjects were passively heated by lower legs immersion in 42 ℃ water, and esophageal temperature (Tes) increased from NT (36.4 ± 0.2 ℃, mean ± SE) to HT (37.4 ± 0.2 ℃). Tes, and skin temperature (Tsk) were measured continuously.
RESULT: Blood flow in ECA was significantly increased during both HT-SIT and HT-SUP compare with NT-SIT by 68 ± 35 and 104 ± 54%, respectively ($P < 0.05$), however there were no effects of postural change and HT on blood flow in CCA, ICA and VA.
CONCLUSION: These data indicated that intracranial blood flow is well maintained during mild-hyperthermia regardless of postural changes.
(COI: No)

P2-363

Experimental validation of teleological hypothesis of cardiolocomotor coupling: effects on gas exchange and muscle deoxygenation during treadmill walking

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The aim of this study was to determine whether cardiolocomotor synchronization (CLS) affects gas exchange efficiency and muscle O_2 utilization during exercise. A healthy elderly subject repeated graded treadmill walking test at constant speed for 20 min. The initial speed and grade were set such that the heart rate intersected the cadence rate. After 3 min warm up the grade was increased 0.5% every 1 min. Minute ventilation (V_E)and O_2 uptake were measured and the ventilatory equivalent for O_2 (V_E/Vo_2) was determined as a measure of gas exchange efficiency. Change in deoxyhemoglobin (Δ[HHb]) at the soleus muscle was sampled by NIRS. CLS was defined as being present when the phase difference between the onset of heartbeat and cadence was fixed over 20 s with the SDs of phase differences being below 0.1. The changes in gas exchange parameters and muscle deoxygenation indices during CLS were evaluated as the differences between the observed and predicted values which were obtained by fitting least squares regression to the desynchronized data. Decrease in V_E/Vo_2 and relative increase in Δ[HHb] were observed during CLS. The reduced V_E/Vo_2 could be accounted for by the reduction of V_E and slight increase in Vo_2. We assume that CLS might decrease the accumulation of ischemic metabolites that are produced during muscle contraction by impeding intramuscular arteries, which would, in turn, act to reduce ventilation. The observation of a relative increase in Δ[HHb] during CLS suggests the increased muscle microvascular O_2 extraction.
(COI: No)

P2-364

Site-specific differential effects of nutrition and exercise on rat musculoskeletal system

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Purpose: Both nutrition and exercise are necessary to maintain musculoskeletal system. This probably is a condition imposed by evolutional necessity for the survival of animals. With this in mind, we studied site-specific effects of nutrition and exercise on the musculoskeletal systems of rats.
Methods: F344 female rats (6 weeks old) were divided into three groups of control (n=10), exercise with dietary restriction (n=6), and exercise without dietary restriction (n=7). Rats of the exercise groups voluntarily ran on a rotary wheel ergometer with a load of 30% of body mass for 8 weeks. The control and exercise without dietary restriction groups were allowed to take diet freely during the experiment period. The exercise with dietary restriction group was allowed to take diet of an amount similar to that of the control group.
Result: Dietary restriction suppressed exercise-induced down-regulation of myostatin with a corresponding increase in muscle mass in plantaris muscle, but not in soleus muscle. Exercise selectively increased the bone volume and mineral density of trabecula in metaphysis. This increase was suppressed by the dietary restriction.
Conclusion: Dietary restriction suppressed exercise-induced growth of musculoskeletal system differentially in a site specific manner. Exercise effects on musculoskeletal system of static function seemed to be more resistant against dietary restriction.
(COI: No)

P2-365

Contribution of EP2 receptor to generation of delayed onset muscle soreness

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We previously demonstrated that nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF) were up-regulated in exercised muscle through activation of B2 bradykinin receptor and up-regulation of cyclooxygenase (COX)-2, respectively, and they sensitized nociceptors resulting in mechanical hyperalgesia (delayed-onset muscle soreness, DOMS). However, the receptor subtypes that bind prostaglandins produced by up-regulated COX-2, has not been identified. Here we examined which prostaglandin receptor subtype was involved in generation of DOMS.
Wild type (WT), EP2 deficient (EP2-/-) and IP deficient (IP-/-) mice were subjected to lengthening contraction (LC). Another group of mice received injection of an EP2 agonist ONO-AE1-259-01 to the lateral gastrocnemius (LGC) muscle. Before and after these treatments the mechanical withdrawal threshold of LGC was evaluated. The change in expression of NGF, GDNF and COX-2 mRNA in the muscle was examined using real-time RT-PCR.
LC induced mechanical hyperalgesia 6-24 h after LC in WT and IP-/- mice, but not in EP2-/- mice. Upregulation of NGF, GDNF and COX-2 mRNA was observed 3 h after LC in WT mice but not in EP2-/- mice. Injecting ONO-AE1-259-01 to the muscle increased expression of COX-2 mRNA.
These results suggest that EP2 contributes to generation of DOMS and EP2 also induces the up-regulation of COX-2 expression.
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P2-366

Regulatory role of VMH-specific Ad4BP neurons in glucose metabolism

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The ventromedial hypothalamus (VMH) plays a crucial role in the regulation of whole body energy homeostasis. Adrenal 4 binding protein (Ad4BP) expressing neurons are limited to the VMH in the hypothalamus. To explore the role of VMH specific Ad4BP neurons on glucose metabolism, we are using Designer Receptors Exclusively Activated by Designer Drugs (DREADD) technology. DREADD is a pharmacogenetic method to activate or inhibit specific neurons in the brain. Upon expression of the stimulatory DREADD 'hM3Dq' or inhibitory DREADD 'hM4Di', a small drug like molecule CNO (clozapine-N-oxide) is used to activate or inhibit neuronal activity, respectively. We implanted VMH targeting bilateral steel cannulas in Ad4BP-cre recombinase transgenic mice and infected double floxed DREADD AAV vectors through it. Brain sections showed expression of DREADD-mCherry only in the VMH. Activation or inhibition of Ad4BP neurons using DREADD system was verified by electrophysiology. We found that Ad4BP-hM3Dq infected mice showed improved glucose clearance during glucose tolerance test (GTT). It also showed increased insulin sensitivity in insulin tolerance test (ITT). In contrast, Ad4BP-hM4Di infected mice had impaired glucose tolerance and decreased insulin sensitivity. Overall, selective activation of VMH Ad4BP neurons improves whole body glucose homeostasis by regulating glucose turnover and increasing insulin sensitivity. Thus, our results elucidate the role of VMH Ad4BP neurons and its importance in regulating whole body glucose metabolism.
(COI: No)

P2-367

Change of sudomotor function evaluated by quantitative direct and axon reflex test with age and sex

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The aim of this study was to quantitatively investigate peripheral sudomotor function through axon reflex-mediated (AXR) and directly activated (DIR) sweating in healthy male (n = 46) and female subjects (n = 48) in the 30-70s groups and to evaluate the correlation between age and sweating function. Quantitative sudomotor axon reflex testing (QSART) with iontophoresis (2 mA for 5 min) and 10% acetylcholine (ACh) were performed to determine AXR and DIR sweating. All experiments were conducted in a thermoneutral condition (temperature, 24.0+/-0.5C; relative humidity, 40+/-3%). The onset time of AXR (r2=0.567) was positively correlated with advancing age, whereas sweat rates of AXR (1) (with iontophoresis) and AXR (2) (without iontophoresis) (r2=0.571, r2=0.486, respectively), DIR (r2=0.594), activated sweat gland density (r2=0.496) and sweat gland output (r2=0.551) were negatively correlated in the two genders with advancing age. Differences between males and females were observed in all age groups. These observations suggest that an attenuation of sudomotor function occurs with ageing due to neurodegeneration resulting from nerve fiber demyelination. Variation in sweating between sexes exists in all age groups

(COI: No)

P2-368

Oligonol supplementation affects sudomotor activity and serum orexin level after heat load in humans

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Oligonol is a low-molecular form of polyphenol, which possesses anti-oxidant and anti-inflammatory activities, and is a potential candidate for an anti-pyretics. This study investigated the effects of Oligonol supplementation on sudomotor activity and serum orexin level, which is related with thermoregulation and energy metabolism, after heat load in 19 healthy male volunteers. We conducted placebo-controlled cross-over trials. Participants took a daily dose of Oligonol 200 mg or placebo for one week. After a 2 week washout period, the subjects were switched to the other study arm. Half-body immersion into hot water (42+/-0.5 C for 30 min), as a heat load, was performed in an automated climate chamber after each supplementation. Tympanic temperature was measured and sudomotor activity was tested in four areas of skin. Serum concentration of orexin (hypocretin) was analyzed immediately after the immersion. Oligonol intake attenuated the increases in tympanic temperature and serum orexn level after heat load, compared with placebo. In addition, Oligonol contributed to the reduction in sweat rate and volume, activated sweat gland density and sweat gland output, but onset time of sweating was the opposite. This study demonstrates that Oligonol supplementation for one week may help human avoid excessive water loss and elevation of body temperature under the heat load.

(COI: No)

P2-369

Required ATP/NAD concentration for the constant ATP production by glycolysis

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Required ATP/NAD concentration for the constant ATP production by glycolysis-Manami Fuchigami, Akira AmanoDepartment of Life Science, Ritsumeikan UniversityConstruction of a mathematical model for cardiac energy production system could help in understanding the cardiac energy production and consumption balance. In this study, we focused on investigating how the glycolysis system works under energy deficiency conditions such as low ATP or high NAD concentrations. We used the glycolysis model proposed by Lambeth et al., which analyzed steady states in two conditions: normal glucose condition (glucose mode) and the condition without glucose (glycogen mode). Result showed that the ATP and NAD concentrations act as ATP production rate controlling factors to glycolysis. The Glycogen mode is superior to the glucose mode. When the ATP concentration is high, the PFK reaction is rate-limiting step. As a result, FBP production comes to stop and glycolysis system doesn't work. In the same way, when the ATP concentration is low, the PK reaction is rate-limited step. To keep high ATP production rate, ATP concentration must be high. To keep high ATP production rate under low glucose or glycogen concentration, a high ATP and NAD concentrations were necessary. When the oxygen level decreases, LDH is extremely important to keep glycolysis activities.

(COI: No)

P2-370

The effect of difference in cooling regions between two exercises on rectal temperature and endurance exercise capacity in the hot environment

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The purpose of this study was to investigate whether difference in cooling region between two exercises affect core temperature and endurance exercise capacity in the hot environment.
Eight adult men volunteered for this study. As a preliminary test, participants were measured in maximal oxygen uptake (VO_2max) by an incremental ramp test with bicycle ergometer. Initially, participants performed a bicycle exercise for 20 min at 60% VO_2max (EX1) and then rested for 15 min on the bicycle ergometer (REST). Subsequently, they performed an incremental ramp test to exhaustion (EX2). They participated in three experiments having three different REST conditions which were non-cooling (CONT), cooling of quadriceps region (QUAD) and cooling of neck region (NECK). Each experiment was separated by at least seven days. All experiments were conducted in hot environment (room temperature: 28.8 ± 0.6 deg C, relative humidity: 60.7 ± 2.0%).
As a result, rectal temperature was no significant difference between those conditions. Subjective parameter by Borg scale was no significant difference between those conditions. Although local cooling did not affect rectal temperature and subjective parameters, exercise time at EX2 in NECK was significantly longer than that in CONT ($p<0.05$). VO_2peak at EX2 in NECK was significantly higher than that in CONT ($p<0.01$). These results suggested that cooling of neck region between two exercise might increase exercise time and VO_2 peak.

(COI: No)

P2-371

Adipose tissue malfunction in prenatally arsenic-exposed mice

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The adverse effects of contaminated arsenic in drinking water have become a concern in developing countries. A possible association between developmental exposure to arsenic and the higher incidence of diabetes has been reported in several epidemiological studies. In our previous study, we have already clarified that the administration of sodium arsenite (85 ppm) in drinking water to pregnant mice from gestational day 8 to 18 leads to impaired energy metabolism in male offspring at 60 weeks of age, such as hyperglycemia, glucose intolerance and insulin insensitivity. In this study, we aimed to test whether impaired energy metabolism in prenatally arsenic-exposed mice is mediated by the malfunction of adipose tissue which contributes to energy metabolism. Firstly, we examined the weight and histology of perirenal fat pad and found an increase of weight with adipocyte hypertrophy in prenatally arsenic-exposed males. According to the increase of fat pad, plasma leptin level was significantly increased. Secondly, we analyzed several gene expressions related to energy metabolism and insulin sensitivity in the fat pad by real-time PCR. Among genes we analyzed, the expression of TNF-α, a cytokine linked to insulin resistance, was significantly up-regulated, indicating the possibility that higher expression of TNF-α impairs insulin action in prenatally arsenic-exposed male. These results suggest that the adverse effect by developmental exposure of arsenic could be mediated by the malfunction of adipose tissue.

(COI: No)

P2-372

Effect of cellular retinol-binding protein II on the enzymatic activity of lecithin:retinol acyltransferase

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Vitamin A is a fat-soluble vitamin needed for many physiological activities. Retinyl esters (REs), animal-derived forms of vitamin A, are hydrolyzed to retinol before entering the absorptive epithelial cells. The retinol thus produced binds to cellular retinol-binding protein (CRBP) II in absorptive epithelial cells, forming a retinol-CRBP II complex. This complex is esterified to REs by lecithin:retinol acyltransferase (LRAT). We asked whether CRBP II affected the amount of REs produced by LRAT. We quantified REs in HEK293T cells overexpressing CRBP II and/or LRAT. Overexpression of LRAT in HEK293T cells led to the accumulation of a large amount of REs in the cells within ten min of addition of retinol to the medium. CRBP II overexpression enhanced the accumulation of REs in LRAT-transfected HEK293T cells.

(COI: No)

P2-373

High-fat diet feeding impaired platelet-derived growth factor receptor alpha (PDGFRα)-mediated up-regulation induced by fasting in NG2-positive oligodendrocyte precursor cells of the hypothalamus in mice

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NG2 cells are thought to differentiate into oligodendrocytes which have ability to maintain myelin structure. Therefore, they are known as oligodendrocyte precursor cells. However, their functions are no only limited as oligodendrocytes: it is suggested that they develop into neurons. It was also discovered that NG2 cells express PDGFR α for their survival. In the present study, we examined whether high-fat diet induced PDGFR α expression in the male mice. The hypothalamus including the ventromedial hypothalamus, the arcuate nucleus, the dorsomedial hypothalamus, and the lateral hypothalamus was dissected as a block. RNA and proteins were extracted. In immunohistochemical study, mice were perfused with 4% paraformaldehyde. Firstly, we confirmed NG2 cells also expressed PDGFR α. Microglia (CD11b, Iba 1), astrocyte (GFAP), and oligodendrocyte (NS, O4) did not expressed PDGFR α. This indicated that PDGFR α-positive cells were NG2 cells and therefore oligodendrocyte precursor cells. Fasting for overnight in the chow diet (control diet) group significantly increased PDGFR α mRNA and its protein. High-fat diet for 10 days also increased PDGFR α mRNA and its protein. However, we found that high-fat diet for 5 weeks did not affect the expression of PDGFR α and β proteins. We suggest from the present study that a high-fat induces inflammation which resulted in dysregulation of PDGFR signals.

(COI: No)

P2-374

Effects of green tea beverage on the spontaneous locomotor activity and intakes of food and water in juvenile mice loaded with isolation stress

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Juvenile stage is susceptible to influences from physical and/or mental stress significantly, irrespective of human and animals. In this study, therefore, we examined whether administered green tea beverage influences the spontaneous locomotor activity of juvenile mice loaded with isolation as mental stress or not. The findings were compared with those obtained from mice bred in a group. Five weeks old-mice were divided into two groups; singly breeding group (three week-isolation stress group; S) and group breeding group (no-isolation stress group). Both group mice were further divided into two groups, respectively; freely water-intake group (W) and freely intake of marketing green tea beverage group (T). Spontaneous locomotor activity of mice was measured as wheel running, fasting blood glucose, oxidative stress, and antioxidant capacity also were measured. Three weeks isolation stress significantly decreased the increasing rate of body weight in W+S group, compared with W group, but not in T and T+S groups. Intake volumes of water and food as well as body weight were decreased with isolation stress. Spontaneous locomotor activity in W+S was decreased, suggesting that isolation influences them. However, no change occurred in the activities in T+S, strongly suggesting that green tea improves lowered locomotion due to the isolation stress, although green tea used in this study significantly increased serum oxidative stress.

(COI: No)

P2-375

The role of TRP channel related to thermal effect during carbon dioxide-rich water immersion

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Carbon dioxide-rich water (CO_2) bathing have been used for balneotherapy of patients with hypertension or peripheral occlusive arterial disease. We have previously reported that CO_2 bathing exerts more thermal effects than fresh water (FR) bathing. Current concepts on modified skin thermoreceptor activities by CO_2 has revealed that the mechanism of lowered a neutral sensation by 2° during CO_2 bathing. Transient receptor potential vanilloid 1 (TRPV1), a member of non-selective cationic channel family expressed in the human sensory neuron, and it has been reported that activation of TRPV1 induces an influx of cations (i.e, Na^+, Ca^{2+}) which are activated by heat above 43° and various chemical agonists such as capsaicin or H^+. Our previous study observed that thermal sensation caused by capsaicin application to the skin is increased by immersion in CO_2 at 33°, suggesting that percutaneously absorbed CO_2 activated TPRV1. However, the mechanism of CO_2 action on TRPV1 has only been partially clarified in humans. The present study examined the role of TRPV1 related to an increase in thermal sensation when immersed into CO_2 of FR at 33° after capsaicin application to the skin. Furthermore, we suggested changes in thermal sensation of immersion into theCO_2 when Capsazepin application to the skin blocked the activity of TRPV1 channels.

(COI: No)

P2-376

Association between energy consumption and behavioral thermoregulation assessed by selection of alternative floor temperatures using mice

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We developed a new experimental apparatus to assess behavioral thermoregulation, involving the selection of two different floor plate temperatures by mice. We used eight ovariectomized adult mice. The experimental box was with two lined hollow stainless floor plates (pl. 1 and 2) of 10 x 20 x 1 cm. The plates were connected to water baths (temperatures, Tpl-1 and -2). The ambient temperature (Ta) was 25℃. We set Tpl-1 and -2 to 30 and 35℃, respectively. We placed a mouse with an implanted temperature telemeter (Tc) in the box and alternated between Tpl-1 and -2 every 5 min and recorded Tc, Ta, and the time staying on the plates once a min for 60 min. We measured oxygen consumption (Vo2) by sampling air in the box. After the measurement on the first day, we fasted the mice and performed measurement once a day on the 2nd and 3rd days. Although the mice stayed on the plate at 35℃ for 58.3 ± 2.3% (mean° SE) of the 60-min experimental duration on the 1st day, the duration increased to 69.0 ± 3.3% on the 3rd day. Tc decreased significantly ($p<0.05$) after fasting. Although Vo2 on the 1st day was 0.7 ± 0.1 L/g (body weight)/min, Vo2 on the 3rd day decreased to 0.6 ± 0.1 L/g/min. At Ta of 25℃, mice stayed on the warmer plates accompanied with decreases of Tc and Vo2, which suggest that the fasted mice decreased energy consumption by activating behavioral thermoregulation. We also performed the same measurements for Ta at 15 and 35℃ (the details were omitted).

(COI: No)

P2-377

β-adrenergic stimulation induces nuclear accumulation of phospho-nuclear kappa B in brown adipocytes of rodents

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Brown adipose tissue (BAT) generates heat in response to sympathetic activation, and is involved in body temperature regulation and body weight control. Previous studies showed that BAT of mice activated by cold exposure or menthol stimulus accumulated phospho-nuclear factor kappa B (pNFκB) in their nucleus. The purpose of this study is to clarify the mechanism of this response. pNFκB was detected by immunohistochemistry (IHC) and by western blot analysis (WB) using anti-pNFκB phosphorylated at Ser276. Mice were kept at 30℃ for 24 h. Half of them were exposed to cold (410℃) for 1h to activate BAT. IHC showed nuclear accumulation of pNFκB in the BAT of mice exposed to cold, but not in the BAT of mice kept at 30℃. WB analysis of whole cell lysates showed a band near 65 kD, corresponding to the known molecular weight of NFκB p65. To clarify the mechanism, experiments were done in primary culture of brown adipocytes. Isoproterenol (Iso), a β-adrenergic agonist, induced nuclear accumulation of pNFκB in cultured brown adipocytes at a concentration range from 10^{-8}M to 10^{-6}M. Notably, this response was evident only in UCP1-positive cells. The response started within 5 min after the Iso application and reached to the maximum level around 10 min. This response was mimicked by forskolin, an activator of adenylate cyclase, and inhibited by H89, an inhibitor of A kinase. These results indicate that nuclear accumulation of pNFκB in BAT occurs through β-adrenergic stimulation followed by the cAMP-A kinase signaling pathway.

(COI: No)

P2-378

Influence of Hepatectomy on Body Temperature Changes in Rats

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The abdominal surgical operation, especially liver resection and transplantation, is known to increase body temperature (BT) during and after surgery, but the precise mechanism(s) are not well understood. Therefore, the aim of the present study was to identify possible mechanisms by which the abdominal surgical operation increases BT using an experimental rat model. Specific pathogen-free male Sprague-Dawley rats, 4 weeks of age, underwent two-thirds partial hepatectomy (PH), one-third splenectomy (PS), or left kidney resection (KR) and rectal temperature (RT) was measured for 5 consecutive days after surgery. RT in PH rats increased and peaked on day 4. However, there was no increase in RT 4 days after PS or KR. In the second part of experiments, we examined the influence gadrinium chloride (GC) and interleukin-1β monoclonal antibody (IL-1β mAb) on the increase in RT following 2/3 PH. Treatment of rats with 20 mg/kg GC or 200 μg IL-1β mAb inhibited the increase in RT induced by PH along with the decrease in IL-1β and prostaglandin E2, which act as pyrogens that change the thermoregulatory set point in the hypothalamus. These results suggest that the abdominal surgical operation, especially liver resection, caused an increase in endogenous pyrogen production, resulting in increased BT.

(COI: No)

P2-379

Application of practical pre-cooling to alleviate thermal strain

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Pre-cooling (i.e., removal of heat from the body immediately prior to exercise) is a popular strategy for alleviating thermal strain and improving exercise performance in hot conditions. Whole body water immersion is the procedure most commonly used to pre-cool in experimental studies. However, the supply of a large volume of water and ice in all field settings is not always possible, or practical. In the present study, we examined the effectiveness of hands and foot water immersion and wearing a cool-vest as practical pre-cooling method on heat strain while wearing protective clothing. Eight males engaged in 60 min of walking at a moderate speed (2.5 km/h) in a hot environment (37℃, 50%relative humidity). Before walking, they immersed hands and foot in water at 18℃ or 28℃ and wore a cool-vest for 30 min. The water was wiped off and the vest was put off, then they wore protective clothings. Rectal temperature increased by 1.0 ± 0.1℃ at the end of the walking in the control trial (without the pre-cooling). The pre-cooling inhibited the increases (18℃, 0.5 ± 0.1℃; 28℃, 0.6 ± 0.1℃, $p<0.05$). In addition, sweat rate, thermal unpleasantness, physical and psychological fatigues were significantly lower in the pre-cooling than in the control trial ($p<0.05$), regardless of water temperature. In 18℃ pre-cooling trial, the changes in heart rate, thermal sensation, and damp sensation were also attenuated ($p<0.05$). Hands and foot water immersion and wearing a cool-vest could be an alternative pre-cooling method alleviating heat strains.

(COI: No)

P2-380

Impairment of cognitive function during passive heat stress

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Excessive elevation of internal body temperature causes a significant strain on either the brain function or the locomotive system. Although hyperthermia impairs psychological and working memory performances, the effect of hyperthermia on cognitive processing remains unknown. We hypothesized that a passive heat stress impairs the cognitive function when the internal temperature was excessively increased. Thirteen healthy males performed an auditory oddball paradigm before and after heat stress (Pre and Post) and when esophageal temperature was increased by 0.8 ℃ and 2.0 ℃ (Mild and Severe). The reaction time and event-related potentials (ERPs) were recorded in these four sessions. As a time control, subjects performed the same sessions without heat stress. The reaction time was shortened while esophageal temperature was elevated relative to the Pre but did not change in the time control trial. However the peak latency and amplitude of N100 did not change throughout the experiment. Although the latency of P300 was unaffected due to heat stress, the amplitude of P300 was significantly reduced at the Severe ($10.5 \pm 5.9\,\mu$V) and Post ($11.1 \pm 5.5\,\mu$V) relative to at the Pre ($16.3 \pm 4.7\,\mu$V). These results suggest that excessive elevation of internal temperature impairs cognitive processing but not auditory processing.

(COI: No)

P2-381

Role of the prostaglandin system in fever following intracranial hemorrhage in mice

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Fever is common after intracerebral hemorrhage (ICH) though its molecular mechanism is unclear. In this study, we established a mouse model of ICH-fever and analyzed the molecular mechanism. To induce ICH, collagenase (Type VII) was injected into the brain under isoflurane anesthesia. When collagenase was injected into the preoptic area, the body temperature started to elevate 30 min after the injection. It then reached the maximum level of 3℃ higher than the baseline between 4 h and 6 h after the injection. Rise in body temperature was positively correlated with the ICH volume. Heat-inactivated collagenase neither induced ICH nor elevated the body temperature. When collagenase was injected into the striatum or pons, ICH was developed but the body temperature did not elevate. Intraperitoneal injection of diclofenac, a non-selective inhibitor of cyclooxygenase (COX), suppressed the elevation of body temperature. On the other hand, NS398, a COX-2 selective inhibitor, did not suppress it. RT-PCR and immunohistochemistry revealed the presence of COX-1, COX-2 and mPGES-1 in the preoptic area after ICH. The present study established a mouse model of ICH-fever, in which COX-1-mediated elevation of prostaglandin (PG), possibly PGE_2, seems to be essential. Although COX-2 is upregulated by ICH, its role in ICH-fever is unclear.

(COI: No)

P2-382

Skin warm/cold threshold during passive heating are attenuated in elderly men

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Autonomic heat dissipative responses are known to be deteriorated with aging, while little is known whether the perception of the increases in skin and esophageal temperatures (T_{sk} and T_{es}) is also deteriorated with aging.

Methods: Seventeen young (21 ± 2 yrs, mean ± SD) and nine elderly (72 ± 3 yrs) healthy men underwent measurements of noticeable increase and decrease (±0.1℃/sec) of skin temperature (warm and cold threshold, respectively) at forearm by using a thermode (6.25 cm^2), and of whole body thermal sensation (VAS) in normothermia (NT; T_{es}, 36.6 ± 0.2℃ in young and 36.5 ± 0.2℃ in elderly, mean ± SE) and mild-hyperthermia (HT; T_{es}, 37.3 ± 0.1℃ in young and 37.5 ± 0.2℃ in elderly; after 40 min of lower legs immersion in 42℃ water). T_{es} and T_{sk} were measured continuously.

Results: Skin warm and cold threshold were blunted in elderly than young men in both NT and HT (all, $P<0.05$). During HT, cold threshold were blunted from NT in young men ($P<0.05$) while it remained unchanged in elderly men ($P=0.97$). Whole body thermal sensation increased during HT from NT in both groups ($P<0.05$), while it showed lower values in elderly than young men during both conditions with a significant difference during NT ($P<0.05$).

Conclusion: Skin warm and cold threshold and also whole body thermal sensation were blunted with aging.

(COI: No)

P2-383

Detection of dynamical human brain temperature changes during tasks and anesthesia

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Human brain temperatures have been measured noninvasively by magnetic resonance spectroscopy (MRS). However, it is not clear whether the brain temperature changes or not during brain activations. We have tried to monitor brain temperature changes precisely during exercises, tongue stimulations and anesthesia (sedation level). The exercises were the lower leg flexion and hand grasp at the rate of about 1 Hz. A capsaicin solution (0.1 %, 20 μL) was used for the stimulation of tongue. The sedation was induced by a single shot intra venous injection of midazolam. Brain temperatures rose monotonously about 0.5 ℃ by 30 min during knee flexion and returned gradually after the end of exercise. The esophagus temperature rose about 0.2 ℃. The net brain temperature change was estimated as 0.01 ℃/min. This temperature rise was found in relatively large regions of the brain. We detected the transient brain temperature falls during light tasks such as hand grasp and tongue stimulation. The brain temperature also fell during sedation. We could estimate the brain energy decrease during sedation as 0.2 W with brain temperature changes. The energy difference between arousal and sedation in our case was about 1 % of the energy that brain needs. Dynamical brain temperature changes could be detected by MRS, and its direction depends on the tasks and maneuvers.

(COI: No)

P2-384

A new portable device to measure sweat rate in hyperthermia for field test

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Sweat rate (SR) in hyperthermia has been measured by a ventilated capsule method. However, this technique is only limited to an experimental chamber. Here, we have developed a portable device to measure SR for the field test. 7 males and 3 females (21-45 yrs) performed cycling exercise for 20-30 min at ~65% peak oxygen consumption rate [30℃ Ta; 50% RH]. SR was measured with a ventilated capsule perfused with dry air at 1.5 l/min on the left chest (SR_{vent}; 12.6 cm^2 area) and a portable device in which 7.5 g of silica gel was contained to absorb water vapor from sweat on the right chest (SR_{pd}; 10 cm^3 volume), while monitoring esophageal temperature (T_{es}). We determined the T_{es} threshold for increasing SR_{pd} (TH_{pd}) and the sensitivity of SR_{pd} in response to increased T_{es} ($\Delta SR_{ad}/\Delta T_{es}$) and those for SR_{vent} (TH_{vent} and $\Delta SR_{vent}/\Delta T_{es}$). T_{es} at rest was ~36.5℃ and increased by 1.4℃ by the end of exercise when SR_{vent} and SR_{pd} increased to ~1.0 mg/min/cm^2 and ~100 μg/cm^2, respectively. Although TH_{pd} was 36.7 ± 0.1℃ significantly lower than TH_{vent} of 37.0 ± 0.1℃, they were significantly correlated ($y = 0.92x + 3.10$, $r = 0.89$, $P = 0.0006$). $\Delta SR_{pd}/\Delta T_{es}$ was $101.5 \pm 23.8\,\mu g/cm^2/℃$, significantly correlated with $\Delta SR_{vent}/\Delta T_{es}$ of 2.0 ± 0.4 mg/min/cm^2/℃ ($y = 0.01x + 0.74$, $r = 0.85$, $P = 0.0017$). Thus, the portable device can be used to measure SR in hyperthermia, suitable for the field test.

(COI: No)

P2-385

Effects of β-amyloid-infusion on behavioral thermoregulation and acquired heat tolerance in rats

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We investigated behavioral thermoregulatory function and ability for acclimating to heat of β-amyloid (Aβ)-infused rats. Male Wistar rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and implanted in the intraperitoneal cavity with a temperature transmitter. A solvent of 35% acetonitrile and 0.1% trifluoroacetic acid (pH 2.0) was used as the vehicle for Aβ peptide (4.9-5.5 nmol). An osmotic pump contained 234 ± 13.9 μl of Aβ solution was subcutaneously implanted in the back and was cannulated into the left cerebral ventricle. Moreover, 0.5 μg of $AlCl_3$ was injected into the right cerebral ventricle with a micro syringe pump. Vehicle-infused rats were used as controls. After 2 weeks, rats were placed in a thermal gradient and their intra-abdominal temperature (T_{ab}) and their ambient temperatures (T_a) selected (T_s) were measured for 3 consecutive days. After the measurements, rats were kept at a T_a of 32℃ for 4 weeks to attain heat acclimation. Then rats were subjected to a heat tolerance test, i.e. they were exposed to a T_a of 36℃ for 3 h. Although there were clear day-night variations of T_s and T_{ab} in all rats, their patterns were significantly altered by Aβ infusion. Moreover, heat acclimation obtained by heat acclimation was attenuated by Aβ infusion. These results suggest that Aβ-infusion in the lateral ventricle modifies behavioral thermoregulation and lowers an ability to acclimate to in rats.

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P2-386

Serum IL-6 and sweating rate responses to passive heating dependent on age

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It is known that sweating responses to heat decrease with age. It is possible that inflammatory cytokines have an effect on sweating responses. We investigated the changes in the serum interleukin 6 (IL-6), the eardrum temperature (Te), and the sweating rate (SR) to the passive heating stress for 60 minutes in younger and elderly men. Three elderly (75.7 ± 3.5yrs) and three younger (23.3 ± 0.6yrs) healthy men participated as volunteers. Changes in the SR of younger men, in response to >0.3 degrees Celsius (℃) of difference between the base line and the Te (DTe), were greater than those of elderly men in response to >0.5℃ in the DTe. The slope of SR against the DTe in the younger men was steeper than that of the elderly men. The concentrations of IL-6 in the younger men were significantly greater than those in the elderly men during the heating stress ($p<0.05$). The concentrations of IL-6 showed little change during the 60 min passive heating in younger men. In contrast they displayed an increasing trend in the elderly. It is possible that the concentrations of IL-6 in the elderly men are higher than those in younger men, and have an effect on the different sweating responses between the two populations.

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P2-387

Reduction of plasma estradiol level affects daily rhythms of body core and tail skin temperature in female rats

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Purpose: We assessed the effect of plasma estradiol (E_2) on daily changes of thermoregulatory responses of the tail vasculature by simultaneous measurements of body core (T_{core}) and tail skin temperature (T_{skin}).
Methods: Female Wistar rats (n=10, age of 8-12 wk) were bilaterally ovariectomized, and placed a telemetry device for the measurements of T_{core} and spontaneous activity (ACT) in the abdominal cavity. T_{skin} was estimated by thermography. Two silicon tubes containing 17-beta estradiol were s.c. placed in one group (n=5, E_2 (+)), and empty tubes for the other (n=5, E_2 (-)). The tubes were removed 10 days after the surgery. T_{core}, ACT, and T_{skin} were measured a day before the tubes removal (PRE), and14 days after (Day 14).
Results: On PRE, T_{core} was higher ($P<0.05$) in the E_2 (-) group than in the E_2 (+) group at 12:30-17:00 (37.2 ± 0.2℃ and 36.5 ± 0.2℃, respectively). T_{skin} was higher ($P<0.05$) in the E_2 (-) group than the E_2 (+) group at 20:00-0:30 (33.4 ± 0.3℃ and 31.2 ± 0.6℃, respectively). On Day 14, both T_{core} and T_{skin} in the E_2 (-) group remained unchanged from the levels of PRE. On the other hand, in the E_2 (+) group, T_{core} became higher than PRE level at 14:30-18:30. T_{skin} was higher than PRE level throughout the day.
Conclusion: A reduction of plasma estradiol may attenuate thermoregulatory response of the tail vasculature to maintain circadian T_{core} rhythm in female rats.

(COI: No)

P2-388

Search of the limbic and cortical regions involved in psychological stress-induced hyperthermia

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Psychological stress-induced hyperthermia is a fundamental autonomic response in mammals. Recently, we have reported that stress induces thermogenesis in brown adipose tissues (BAT), hyperthermia and tachycardia by activating a neural pathway from the dorsomedial hypothalamus (DMH) to the medullary raphe. However, the upper neurons that provide the DMH with stress signals to drive the stress responses are unknown. To identify such upper neurons, in this study, we performed *in vivo* electrophysiological experiments using anesthetized rats, in which neurons in several forebrain regions implicated in emotion were stimulated with nanoinjections with bicuculline, a $GABA_A$ receptor antagonist, and the effects on sympathetic effectors were examined. Nanoinjection of bicuculline into either the medial prefrontal cortex (mPFC) or the lateral septal nucleus (LS) increased BAT sympathetic nerve activity, BAT thermogenesis and heart rate. These evoked sympathetic responses were diminished by inhibition of bilateral DMH neurons with muscimol nanoinjections. Inhibition of bilateral mPFC neurons with muscimol injections in free-moving rats strongly reduced BAT thermogenesis and hyperthermia induced by exposure of the rats to social defeat stress, a sociopsychological stress model. In contrast, inhibition of LS neurons exhibited a weaker inhibitory effect on these stress responses. These results suggest that the mPFC is involved in psychological stress-induced BAT thermogenesis and hyperthermia through activation of DMH neurons.

(COI: No)

P2-389

Pleasantness induced by local thermal stimulus may be related to the activity of the frontal cortex: assessment by near-infrared spectroscopy

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Purpose: Thermal stimuli to body core and surface can evoke pleasantness, although the neural mechanisms are not fully understood. In the present study, we assessed blood oxygenation level dependent (BOLD) signals of the frontal cortex by NIRS, when human subjects had local skin heating or cooling during whole-body warming or cooling.
Methods: Ten healthy subjects had local thermal stimulus of 41℃ or 17℃ during whole-body stimulus of 45℃, 33℃, or 24℃. The local stimuli were delivered on the left forearm with the Peltier device, and the whole-body stimuli were conducted by wearing a water-perfusion suit. A subject sat on chair at least until the skin temperature became stable, the local skin stimulation with either temperature was conducted four times. During the period, the BOLD signals were obtained by NIRS, and the subjects were asked to report thermal sensation and pleasantness using visual analogue scale (VAS) in the last session.
Results: There were no differences in local thermal sensation among the three whole body temperatures, although the local thermal pleasantness was different. When the thermal unpleasantness in the forearm was augmented, the BOLD signal decreased in the frontal cortex.
Conclusion: NIRS could be a tool to assess thermal pleasantness induced by local thermal stimuli.

(COI: No)

P2-390

Effect of systemic estradiol administration on tail-hiding behavior and cFos expression of brain areas in female rats in the cold

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INTRODUCTION: Rats place their tails underneath their body trunks in the cold (tail-hiding behavior), which may be a thermoregulatory behavior. The aim of the present study was to test the effect of estradiol (E_2) on tail-hiding behavior and neural activity in brain areas in the cold.
METHODS: Ovariectomized rats were implanted a silastic tube with or without E_2 (22.3mg) underneath the dorsal skin (E_2(-) and E_2(+) groups), and exposed to 27℃ or 10 or 16℃ for 2-h with continuous body temperature (T_b), tail skin temperature (T_{tail}), and tail-hiding behavior measurements. cFos immunoreactive (cFos-IR) cells in the insula, secondary somatosensory cortex, medial preoptic nucleus, parastrial nucleus, amygdala, lateral parabrachial nucleus were counted in four segments: seg1, 2, 3, and 4 (bregma -0.36, -1.44, -2.64, and -9.00 mm), respectively.
RESULTS: T_b and T_{tail} were not different between the E_2(-) and E_2(+) groups. Only at 16℃, the duration and the onset of tail-hiding behavior in the E_2(+) group were higher than that in the E_2(-) group. Only at the insula in seg2 at 16℃, cFos-IR cells in the E_2(-) group were greater than that in the E_2(+) group.
CONCLUSION: E_2 might modulate a tail-hiding behavior of female rats in a mildly cold, and the insula may relate to the response.

(COI: No)

P2-391

Possible role of hypothalamic FABP7 in the control of glial cell proliferation and food intake

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Introduction: A growing evidence suggests that hypothalamic fatty acid sensing is associated with regulation of systemic energy balance, including insulin secretion, adipose deposition and food intake. We have so far reported that fatty acid binding protein 7 (FABP7) is highly expressed in astrocytes and oligodendrocyte precursor cells (OPCs) and functionally involved in their proliferation. In this study, we examined the detailed localization of FABP7 in the hypothalamus including the arcuate nucleus (ARC) and median eminence (ME). Furthermore, we sought to explore the role of FABP7 in the hypothalamus through the phenotypic analysis of FABP7 KO mice under high fat diet food feeding.
Method and Results: In immunohistochemistry, 70% of FABP7+ cells in the ARC and ME were revealed to be NG2+ cells, and 30% of FABP7+ cells were GFAP+ cells. When the hypothalamic cells were labeled with bromodeoxyuridine (BrdU) by drinking water or interventricular injection, approximately 80% of BrdU+ cells in the ARC of wild-type (WT) mice were FABP7+ cells, but the significant decrease in the density of BrdU+ cells was detected in the ARC of FABP7 KO mice. In addition, FABP7 KO mice fed with high fat diet (HFD) showed the significant decrease in their HFD food intake as well as their body weight gain compared with WT mice.
Conclusion: Fabp7 regulates proliferation of NG2+ cells in hypothalamic ARC and ME. The role of FABP7 in the control of neuronal activity in hypothalamus and its possible involvement in the regulation of HFD food intake were highly suggested.
(COI: No)

P2-392

DGKε deletion induces lipid metabolism impairment and adipose tissue insulin insensitivity

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Triglyceride (TG) synthesis and breakdown are closely related to glucose homeostasis, which is regulated by insulin. TG is synthesized from diacylglycerol (DG) by the action of DG acyltransferase. DG is also known to activate novel PKC (nPKC) leading to interruption of appropriate insulin signaling, i.e. insulin resistance. These facts suggest that DG metabolism is critical in insulin signaling and TG synthesis. DG kinase (DGK) is an enzyme that converts DG to phosphatidic acid. Previously, we demonstrated that high fat diet (HFD) induces lipid accumulation and glucose intolerance in DGK ε -KO mice. In this study, we investigated detailed mechanisms for these phenotypes. To this end, HFD-fed DGK ε -KO mice were examined by glucose and insulin tolerance tests. We also measured serum insulin, TG and free fatty acid levels, and performed histological and immunoblot analyses. In DGK ε -deficient adipose tissue, phosphorylation level of PKC θ was increased whereas that of Akt was attenuated. These results suggest that DGK ε depletion results in PKC θ activation and Akt inactivation under HFD feeding, which may lead to impaired insulin signaling. Furthermore, we found that protein levels of adipose triglyceride lipase (ATGL) and its transcription factor FOXO1 were also attenuated, suggesting that DGK ε deficiency downregulates lipolysis in adipose tissue. On the other hand, insulin signaling was kept intact in other organs, such as liver and skeletal muscle. These results suggest that DGK ε -KO mice show dysregulation of lipid metabolism and impaired insulin signaling in adipose tissue.
(COI: No)

P2-393

Global gene expression profiling of the inhibitory effect of estrogen on the cell proliferation of MDA-MB-231 breast cancer cells stably transfected with estrogen receptor

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Treatment with estrogen leads to the inhibition of proliferation of estrogen receptor (ER) negative breast cancer cells stably transfected with ER α cDNA. The elucidation of the ER-mediated inhibitory mechanism will provide a novel strategy for the down regulation of breast cancer growth. In this study, the ER negative MDA-MB-231 breast cancer cells were stably transfected with ER α cDNA and several clones (MDA-ERs) were established. Although the cell proliferation of all the clones was inhibited by the treatment with estrogen for longer times, 24 h estrogen treatment inhibited cell proliferation of MDA-ER#3 but not of MDA-ER#1 clone, despite the same genetic background of the two clones. To compare the gene expression levels of MDA-ER#3 with MDA-ER#1, we applied cDNA microarray analysis to those two clones treated with or without estrogen for 24 h. In each clone, the genes which expressions were altered more than twice by treatment with estrogen were identified as up-regulated or down-regulated genes. Among the up-regulated genes, 119 genes exhibited 142-465% higher estrogen-responsiveness in MDA-ER#3 than that in MDA-ER#1. Among the down-regulated genes, 14 genes exhibited 40-69% lower estrogen-responsiveness in MDA-ER#3 than that in MDA-ER#1. These differentially regulated, estrogen-responsive genes included proliferation-related genes, which may play an important role in the antiproliferative effect of estrogen.
(COI: No)

P2-394

wx/ae rice has effects of improving hyperlipemia and fatty liver induced by high-fat diet

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wx/ae rice includes more resistant starch and γ -Oryzanol than Koshihikari which have effects of reducing the lipid in the blood. In this study, male C57BL/6J mice aged 8 weeks were fed of chow diet (10% kcal), high-fat diet (45% kcal), high-fat diet + wx/ae brown rice, a high-fat diet + Koshihikari brown rice, for 12 weeks. The powder of brown rice was contained 30%. In the high-fat diet + wx/ae brown rice group, feces amount is increased to 1.5 times, triglycerides content of feces per 1g was more than double compared to the high-fat diet group. The high-fat diet + wx/ae brown rice group, triglycerides and cholesterol in the blood are reduced to 50% compared to high-fat diet group, and became a concentration similar to the normal diet group. In addition, after feeding high-fat diet for 8 weeks, these mice divided 3 groups as follows: chow diet, high-fat diet + Koshihikari brown rice, high-fat diet + wx/ae brown rice were switched for 4 weeks. The fatty liver is improved by switching to high-fat diet + wx/ae, not Koshihikari brown rice. These results suggested that wx/ae brown rice has the effect of improving hyperlipemia and fatty liver by egestion of lipid to feces.
(COI: No)

P2-395

Expression of purinergic receptors on mouse brown adipocytes

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Neurotransmitter receptors on brown adipocytes and sympathetic nerve fibers contribute to thermogenesis by mediating Ca^{2+} dynamics among brown adipocytes. We investigated the functional expression of purinergic receptor subtypes on brown adipocytes of mouse interscapular fat. Ca^{2+} imaging showed that applied 10 μM ATP, 10 μM BzATP (a $P2X_1$, $P2X_7$ and $P2Y_1$ agonist), 1 μM 2MeSATP (a $P2Y_1$ and $P2Y_{11}$ agonist) or 100 μM UTP (a P2Y agonist) increased intracellular Ca^{2+} concentration. RT-PCR suggested the expression of $P2X_1$, $P2X_3$, $P2X_4$, $P2X_5$, $P2X_7$, $P2Y_1$, $P2Y_2$, $P2Y_6$, $P2Y_{13}$ and $P2Y_{14}$ among the seven P2X subtypes and seven P2Y subtypes examined. Immunoblotting confirmed the expression of $P2X_1$ and $P2X_7$. These results showed the functionally expression of $P2X_1$ and $P2X_7$ on mouse brown adipocytes. The roles of purinergic receptor subtypes in the thermogenesis are discussed.
(COI: No)

P2-396

Gene Expression Analysis of immune cells in response to Nucleoprotein

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Salmon soft roe (Shirako) contains in plenty nucleic acids and protamin protein, termed as Nucleoprotein (NP). Previously we showed that crude NP derived from food and also purified DNA components affect on immune cells to express some IL-8 and induce differentiation into mature immune cells. We did further analysis focus on the stimulation mechanism of immune cells in response to purified DNA components by Micro Array Analysis and subsequently IPA Analysis. As a result, 62 and 109 genes were up and down-regulated, respectively. Here, we suggest that purified DNA component taken from food affect on immune cells to induce some cytokines and increase mucosal immunity which contribute to our health and anti-aging process.
(COI: No)

P2-397

Effect of intermittent sucrose intake for several weeks on central glucose-sensing system in mice

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The central glucose-sensing system has neurons to respond to glycemic levels and regulates feeding behavior. Intermittent sucrose intake under food deprivation induces overconsumption of sucrose. We previously reported that the feeding pattern enhances glucose tolerance and attenuates the anorectic effect of systemic glucose injection in mice. However, it remains unclear whether the intermittent sucrose intake changes the glucose-sensing system. Here, we investigated the effect of intermittent sucrose intake on neuronal responses to systemic glucose injection. Mice under 20-h food deprivation received 4-h access to chow with or without sucrose solution (FD/Suc and FD mice, respectively) for 24 days. Sucrose intake of FD/Suc mice was greater than water intake of FD mice after Day 2. After Day24, the mice were food deprived overnight followed by an intraperitoneal glucose injection. Then, the mice were perfused 90 min after the injection and the brains were processed for Fos-immunoreactivity. The number of Fos positive cells in the lateral hypothalamus and perifornical area (LH/PFA) in FD/Suc mice were smaller than those in FD mice. This finding demonstrates that the intermittent sucrose intake under food deprivation attenuates the neuronal responses to systemic glucose injection in the LH/PFA. It is suggested that the change in the central glucose-sensing system contributes to overconsumption of sucrose.

(COI: No)

P2-398

Effect of postprandial chewing gum on diet-induced thermogenesis

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The present study was to examine the effect of postprandial chewing gum on diet-induced thermogenesis (DIT). After baseline measurements for 20 min in the overnight fasting state, twelve healthy normal-weight males fractured a 620-kcal test meal for as fast as they could. In the chewing gum (RG) trial, they started chewing a 3-kcal gum immediately after meal, and chewed the gum for 15 min. In the no-chewing gum (RN) trial, they ingested 3 kcal of sugar with test meal instead of the gum. DIT was calculated from oxygen uptake and body mass, and was recorded until 180 min after meal. Duration and the number of chews during test meal showed no significant differences between the RN trial and the RG trial (304 ± 32 vs. 298 ± 26 s, 238 ± 20 vs. 237 ± 24 times, RN vs. RG, respectively, $p>0.05$). The number of chews for 15-min chewing gum (RG trial) was 836 ± 51 times. DIT was significantly greater in the RG trial than in the RN trial (14 ± 4 vs. 20 ± 5 kcal / 180 min, RN vs. RG, $p<0.05$). Effect of postprandial chewing gum on DIT was observed until 45 min after meal. Previous study reported that energy expenditure by solely chewing gum for 12 min (chewing frequency; 100 times / min) was 11 kcal, and increased energy expenditure returned to baseline levels immediately after cessation of chewing (Levine 1999). In the present study, postprandial chewing gum had an influence on DIT until 45 min even after chewing gum. These results suggest that 15-min chewing gum immediately after meal increases DIT.

(COI: No)

P2-399

SIRT1 in the central nervous system regulates food preference

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In order to address the role of SIRT1 in the food preference regulation, we either over-expressed or knocked-out SIRT1 in all neurons by crossing Tau-Cre mice with either Rosa26-Sirt1 mice or Sirt1-flox mice, and measured their food preference. SIRT1 overexpression increased preference for high-fat diet, whereas SIRT1 knock-out increased preference for high-sucrose diet. Expression analysis of hypothalamus, ventral tegmental area, nucleus accumbens, and prefrontal cortex of these mice fed normal chow or high-fat diet revealed that SIRT1 overexpression suppressed the expressions of tyrosine hydroxylase (Th) and dopamine transporter (Dat) and increased the expression of oxytocin (Oxt) in the hypothalamus. Next, we either overexpressed or knocked-down Sirt1 in hypothalamic N38 and N41 cells and checked the effect on the expressions of Th, Dat, and Oxt. We found that SIRT1 regulates the expression of these genes accordingly. Dopamine system is known to promote fat preference, whereas oxytocin is known to suppress carbohydrate preference. Therefore, Sirt1 in the central nervous system may regulate food preference through the modulation of dopamine and oxytocin system.

(COI: No)

P2-400

Glucagon directly activates vagal afferent neurons: possible role in feeding regulation

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Background and Aim: It has been reported that glucagon is transiently secreted immediately after meals and implicated in meal-evoked satiety. Intraperitoneal injection of glucagon reduces feeding, and this effect is attenuated by subdiaphragmatic vagotomy, suggesting the involvement of the vagal afferent nerves. However, the mechanism by which glucagon influences vagal afferents is less defined. In this study, we investigate the direct action of glucagon on vagal afferent nodose ganglion (NG) neurons.
Results: Glucagon receptor mRNA was detected in mice NG using by RT-PCR. Glucagon at 10^{-9}-10^{-7} M, but not 10^{-10} M, increased cytosolic Ca^{2+} ($[Ca^{2+}]_i$) in isolated single NG neurons. Glucagon at 10-8 M exerted a maximal effect, inducing $[Ca^{2+}]_i$ increases in approximately 8% of NGNs. Glucagon-induced $[Ca^{2+}]_i$ increases were attenuated by a glucagon receptor antagonist. All of the glucagon-responsive NG neurons exhibited $[Ca^{2+}]_i$ responses to cholecystokinin-8 (CCK-8), a hormone known to reduce food intake via direct interaction with vagal afferents.
Conclusion: These results demonstrate that glucagon directly interacts with the subpopulation of vagal afferent neurons that respond to CCK-8. This interaction may underlie the production of satiety after meals. This study also suggests that glucagon and CCK-8 share a common vagal afferent-mediated pathway that inhibits feeding.

(COI: No)

P2-401

Peripheral oxytocin directly activates vagal afferents to decrease food intake

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Oxytocin (Oxt), produced in the paraventricular nucleus and supraoptic nucleus of hypothalamus, regulates feeding. Peripheral administration of Oxt suppresses feeding and ameliorates obesity. However, the route through which peripheral Oxt informs the brain is unclear. We investigated whether vagal afferents mediate the sensing and anorexigenic effect of peripherally injected Oxt in mice. Oxt evoked action potential firings and increased cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in single vagal afferent neurons. The Oxt-induced $[Ca^{2+}]_i$ increases were inhibited by Oxt receptor antagonist. Intraperitoneal injection of Oxt decreased feeding and increased c-Fos expression in the nucleus tractus solitarius (NTS) of medulla, to which vagal afferents project. This feeding suppression and c-Fos expression in NTS were blunted by subdiaphragmatic vagotomy. In obese diabetic *db/db* mice, leptin failed to but Oxt increased $[Ca^{2+}]_i$ in vagal afferent neurons, and single injection or sub-chronic infusion of Oxt decreased feeding and body weight gain. These results demonstrate that peripheral Oxt injection suppresses feeding by activating vagal afferents, and that this "peripheral Oxt-vagal afferents-brain" axis is effective for treating hyperphagia and obesity.

(COI: No)

P2-402

Accumulation of vitamin A and radiocontamination of arctic animals

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We have performed a systematic characterization of the hepatic vitamin A storage in mammals and birds of the Svalbard archipelago and Greenland. The top predators including polar bear, arctic fox, bearded seal and glaucous gull contained about 10-20 times more vitamin A than all other arctic animals studied as well as their genetically related continental top predators. This massive amount of hepatic vitamin A was located in large lipid droplets in hepatic stellate cells (HSCs). The droplets made up most of the cells' cytoplasm. The development of such an efficient vitamin A-storing mechanism in HSCs may have contributed to the survival of top predators in the extreme environment of the arctic. The HSC that has capacity of taking up and storing a large amount of vitamin A plays pivotal roles in maintenance of food web, food chain, biodiversity, and eventually ecology of the arctic. In March 2011, the big earthquake and tsunami attacked the east coast of Northern Japan. Radiocontamination was caused by the damage of Fukushima power plant. We visited Svalbard in August-September 2011 and August-September 2013 and examined radiocontamination in fauna and flora of the arctic. We would like to discuss based upon the data obtained.

(COI: No)

P2-403

Change metabolic enzymes activity and effects of chaga mushroom (*Inonotus obliquus*) in diabetes mellitus

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Introduction: It was thought that type II diabetes mellitus was induced by impaired insulin secretion and through inhibiting effects of insulin by glucose tolerance factor. But, it was not known very well how various metabolic enzymes were affected by diabetes mellitus (DM).
Objectives: The changes of metabolic enzymes activities caused by DM are analyzed. Then, effects of chaga mushroom well known as antioxidant factors are estimated to make clear the anti-diabetes activity.
Materials and methods: Streptozotocin (STG) sol was administrated by intra-peritoneal injection into SD rat induce DM. Then, various metabolic enzymes activities were analyzed by apiRZYM system (SYSMEX bioMeneux Co. Ltd, Tokyo) in DM rats. Still more, the metabolic enzyme activities of chaga administrated rats were similarly analyzed to clarify the anti-diabetes activity.
Results and discussion: High blood-sugar and cholesterol levels and low levels of lipid metabolic enzymes were detected were detected in DB rats induced by STG. Especially, it was made clear that β-galactosidase activity as an aging factor increased in DB rats. Still more, It was seemed that these changes were inhibited by chaga extracts.
Conclusion: High blood-sugar and cholesterol levels and β-galactosidase activity increased in DB rats induced by STG. It was made clear that these changes were inhibited by chaga as amti-diabetes.

(COI: No)

P2-404

Global gene expression analysis to identify molecular mechanisms that enable hibernation in mammals

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Hibernation is a state during which drastic metabolic suppression occurs to survive severe winter condition with little or no food. Mammalian hibernators must achieve adaptive remodeling in tissues and cells to hibernate without severe tissue injuries or massive cell death. Interestingly, several reports suggested that the adaptive remodeling is not observed in non-hibernating seasons but is induced in the pre-hibernation periods. However, the molecular and cellular mechanisms for the adaptive remodeling remain to be poorly understood. To reveal this, we have conducted global gene expression analysis with RNAseq by using syrian golden hamster (Mesocricetus auratus), which initiates hibernation under short day and cold acclimation condition after prolonged periods (about 4~12 weeks). We have examined several organs, including liver, skeletal muscles, white adipose tissues and brown adipose tissues, that are involved in energy homeostasis at the systemic level. This analysis have identified genes that are specifically up-regulated or down-regulated during hibernating periods. Possible contribution of those genes to the establishment of adaptive mechanisms for hibernation will be discussed.

(COI: No)

P2-405

Exploring adaptive mechanism in adipose tissues that enables animals to survive hibernation periods

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Hibernation is an energy-saving behavior to survive during winter with little or no food. During hibernation, animals experience dramatic decreases in core body temperature, heart rate and oxygen consumption, which require adaptive remodeling in tissues and cells for the animals to survive without tissue injuries or cell death. Interestingly, the adaptive remodeling seems to be mainly induced before the entrance to hibernation, because it is not observed in non-hibernating season. However, molecular mechanisms responsible for the adaptive remodeling in tissues and cells remain largely unclear. In this study, we investigate the remodeling mechanism of brown and white adipose tissues by utilizing syrian golden hamster (Mesocricetus auratus), which initiates hibernation under short day and cold acclimation condition after prolonged periods (about 4~12 weeks). To reveal molecular and cellular mechanisms for adaptive remodeling for hibernation, we have conducted global gene expression analysis with RNAseq and revealed that expression of several genes related to development of adipose tissues, lipid metabolism and synthesis, and non-shivering thermogenesis, changes during hibernation periods, which may contribute to the establishment of adaptive energy metabolism and thermogenesis for hibernation.

(COI: No)

Poster Presentations

Day 3

（March 23, 12:15～13:30）

P3-001～P3-019	Neuronal projection
P3-020～P3-065	Neurohistochemistry, Neurochemistry
P3-066～P3-085	Autonomic nervous system
P3-086～P3-117	Higher brain function
P3-118～P3-135	Motor function
P3-136～P3-206	Sensory function, Sensory organs
P3-207～P3-248	Neurological disorders, Neuropathophysiology
P3-249～P3-269	Others of Neuroanatomy, Neurophysiology, Neuronal cell biology
P3-270～P3-302	Behavior, Biological rhythm
P3-303～P3-336	Gross anatomy
P3-337	Anthropology
P3-338～P3-353	Pathophysiology
P3-354～P3-362	Drug Effect
P3-363～P3-376	Medical education
P3-377～P3-390	Others

P3-001

Glutamatergic circuits in the song system of zebra finch brain determined by gene expression of vGluT2 and glutamate receptors

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The songbird brain has a system of interconnected nuclei that are specialized for singing and song learning. Electrophysiological findings indicate a role for the glutamatergic neurons in the song system. Vesicular glutamate transporter 2 (vGluT2) is considered to be a specific biomarker of glutamatergic neurons in birds. Neurons receiving glutamatergic afferents express mRNA of ionotropic glutamate receptor subunits. This study examined expression of vGluT2 and glutamate receptor subunit mRNAs in nuclei of the song pathways of male zebra finch brain by in situ hybridization. VGluT2 mRNA was revealed high density of expression in the song nuclei, namely HVC, lateral magnocellular nucleus of the anterior nidopallium, and robust nucleus of the arcopallium. Area X did not show expression of vGluT2 mRNA. Nuclei in the descending motor pathway (dorsomedial nucleus of the intercollicular complex and retroambigual nucleus) were expressed vGluT2 mRNA. Target nuclei of vGluT2 mRNA-expressing nuclei showed hybridization signals for mRNAs of ionotropic glutamate receptor subunits. At least one of five subunit mRNAs (GluA1, GluA4, GluK1, GluN1, GluN2A) was expressed in song nuclei. The present findings support the existence of glutamatergic circuits in the song system in songbirds.

(COI: No)

P3-002

Prenatal valproic acid exposure induces aberrant distribution of spinal nerves in mice

Juramt, Bold; Sakata, Hiromi; Fukui, Yoshihiro (*Department of Anatomy and Developmental Neurobiology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima Japan*)

Background: Teratogenicity of anticonvulsant valproic acid (VPA) is well known and has a potential to induce congenital malformations, such as a neural tube defect, in fetuses whose mothers are treated with it during pregnancy. However, the detailed mechanism of VPA teratogenicity still remains unknown. Thus, we examined the effects of VPA on peripheral nerve fiber innervation during development using a mouse model.
Methods: A single dose of 400 mg/kg VPA was subcutaneously injected in pregnant ICR mice on any one gestational day (GD) 6 to GD 9. On GD 10, embryos were collected from the pregnant mice and quickly immersed into 4% paraformaldehyde/0.1M PB (pH7.2). Distribution of neurofilament-immunoreacitve nerve fibers in the embryos was examined using a whole-mount immunostaining technique.
Results: The whole-mount immunostaining was clearly detected as nerve fiber bundles that consist of both cranial and spinal nerves. There was no obvious change in distribution of cranial nerves. On the other hand, abnormal distributions of spinal nerves, such as a loss of a whole bundle and an intrusion into adjacent segments, were identified in prenatal VPA-exposed embryos. The incidence of aberrant spinal nerve was higher in the embryos exposed to VPA on GD 8.
Conclusions: We demonstrated that prenatal VPA exposure has a potential to induce aberrant distribution of spinal nerves and development of spinal nerve innervation could be more susceptible to VPA exposure on GD 8 in mice.

(COI: No)

P3-003

A newly-identified hypothalamic area enriched with perineuronal nets

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Perineuronal nets (PNNs) are specialized extracellular matrix structures in the adult brain that play important roles in regulating synaptic plasticity. In this study, we examined the formation of PNNs in the mouse hypothalamus using WFA lectin (a broad PNN marker), and found a previously unidentified region located between the fornix and paraventricular nucleus (PVN). We named this new region the "hypothalamic delta area" (HDA), referring to its triangular shape. DNA microarray experiments and histochemical studies identified at least two types of neurons in the HDA: enkephalin- and calretinin-positive neurons, both of which were GABA negative. Furthermore, the HDA was shown to have bidirectional neural connections with the lateral septum (LS) and intra-hypothalamic nuclei such as the ventromedial hypothalamic nucleus and dorsal part of the premamillary nucleus. We also confirmed enkephalinergic projections from HDA neurons to the LS, and inversely, calbindin-positive LS neurons as afferents to the HDA. c-Fos expression analysis revealed that the activity of HDA neurons were increased by emotional stressors such as open field test, restraint stress, and aggressive behavior, but not by the metabolic stressors such as fasting and dehydration. These results suggest that the HDA is a newly-identified hypothalamic area connecting the hypothalamus with the limbic system and has specific functions related to emotional stressors.

(COI: No)

P3-004

A new reporter rat line which conditionally expresses red fluorescent protein (tdTomato)

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Rats offer potential advantages of larger body size and progressed ability to accomplish complex behavioral tasks over mice. However, the conditional gene expression system has not been enough arranged despite of its importance in anatomy and physiology. Here we report a conditional reporter rats which express red fluorescent protein (tdTomato) under Cre/loxP recombination system. The effectiveness of conditional expression was verified by the following three evidences. (1) When Cre was expressed in striatum, hippocampus or cerebellum using AAV, each Cre-immuno-positive cells merged with tdTomato signals. (2) When Cre expression was targeted specifically in the cortical layer 2/3 using *in utero* electroporation at embryonic day 18 (E18), neurons in the layer 2/3 were visualized by tdTomato fluorescence. (3) When the double transgenic rats with *phox2b*-Cre and *floxed* tdTomato were examined at embryonic E12.5, tdTomato was expressed in neurons of several hindbrain regions that are involved in the autonomic nervous system as well as in neurons that are responsible for respiratory rhythm generation, and co-localzed with endogenous Phox2b proteins. The neuronal fiber projections were clearly visualized by the tdTomato signals. Our reporter rat would facilitate the neurophysiological studies and the connectomics of identified neurons which express Cre under a certain promoter.

(COI: No)

P3-005

In vivo calcium imaging of thalamocortical axons in mouse primary visual cortex

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The prominent aspect of primary visual cortex (V1) is the response selectivity to visual stimulus features. Layer 4, the major entrance layer of V1, receives main visual input from two thalamic nuclei, lateral geniculate nucleus (LGN) and lateral posterior nucles (LPN). Layer 4 excitatory neurons in mouse V1 possess high selectivity for stimulus orientation. Recent studies indicated that LGN axons targeting layer 1, another recipient layer of thalamic input, had sharp orientation selectivity (Cruz-Martin et al., 2014). However, it is still unknown whether layer 4 also receives orientation tuned input from thalamus. To answer this question, we performed in vivo two-photon calcium imaging of thalamocortical axons in mouse V1 and investigated their response selectivity to visual stimuli. For axonal calcium imaging, GCaMP6s, genetically encoded calcium indicator, was locally expressed in either LGN or LPN neurons by AAV-mediated method. Boutons of thalamocortical axon expressing GCaMP6s were clearly visible in layers 1 through 4 of V1 and we could record their response selectivity to visual stimuli. We found LGN axons arborizing in layer 4 had broad orientation selectivity, while LPN axons in layer 4 had mixture of sharp and broad orientation tuning. These results suggest that layer 4 neurons also receives orientation tuned input form LPN, while largely untuned input from LGN.

(COI: No)

P3-006

Projection from lateral habenula to trigeminal mesencephalic nucleus and its function

Ohara, Haruka; Tachibana, Yoshihisa; Sato, Fumihiko; Takeda, Rieko; Oka, Ayaka; Kato, Takafumi; Yoshida, Atsushi (*Dept. of Oral Anatomy and Neurobiology, Grad. Sch. Dent. Osaka Univ., Osaka, Japan*)

The lateral habenula (LHb) is implicated in disappointment and expectation of negative conditions such as stressful conditions, suggesting that it is also involved in motor control of food intake. The trigeminal mesencephalic nucleus (Vmes) neurons convey deep sensations from masticatory muscles and periodontal ligaments, and function in orofacial movements, especially jaw movements. Therefore, we examined whether LHb neurons activated by stress to the animals directly project to Vmes neurons in rats. After a retrograde tracer, FG, was injected into Vmes, many neurons were labeled bilaterally in both the lateral part (LHbL) and medial part (LHbM) of LHb. After injections of an anterograde tracer, BDA into LHb, axon fibers and terminals were labeled bilaterally in Vmes. Some BDA-labeled terminals contacted the cell bodies of Nissl-stained Vmes neurons bilaterally. After FG injections into Vmes and subsequent application of restraint stress, many c-Foss immunoreactive (ir) cells were observed bilaterally in LHb; the number of c-Foss-ir cells in LHbM was higher than that in LHbL bilaterally. A small number of FG/c-Fos double labeled neurons were found bilaterally in LHb; the number of double labeled neurons in LHbM was slightly higher than that in LHbL. The ratio of double labeled neurons to FG labeled neurons in LHb was higher than that found in control cases with FG injections into Vmes but no restraint stress. This study suggested that LHb neurons activated by stress directly project to Vmes neurons.

(COI: No)

P3-007

Central processing of masticatory muscle sensation

Fujio, Takashi; Sato, Fumihiko; Tomita, Akiko; Ikenoue, Etsuko; Haque, Tahsinul Md.; Yoshida, Atsushi (*Dept. of Oral Anatomy and Neurobiology, Grad. Sch. Dent. Osaka Univ., Osaka, Japan*)

The masticatory muscle sensation is involved in the orofacial movements. This sensation is conveyed to the supratrigeminal nucleus (Vsup) by the trigeminal mesencephalic nucleus neurons in cats. However, little is known about how to identify the Vsup and about the central processing of the sensation through the Vsup. To address these issues, we used neuronal tract tracing and electrophysiological recording techniques in the rat. After application of cholera toxin subunit B to the masseter nerve (MN), we found anterogradely labeled axon terminals in almost entire area of the Vsup, which was cytoarchitectonically identified. The Vsup was also identified electrophysiologically by recording responses to electrical stimulation of the MN and the passive jaw-jerk; no fast responses were recorded after electrical stimulation of the lingual nerve. After injections of biotinylated dextranamine into the Vsup, anterogradely labeled axon terminals were found contralaterally in the caudo-ventromedial part of the ventral posteromedial nucleus (VPMcvm) and bilaterally in the paracentral nucleus in the thalamus. The VPMcvm was also identified electrophysiologically by recording responses to electrical stimulation of the MN and the passive jaw-jerk. After Fluorogold injections into the VPMcvm, retrogradely labeled cells were found contralaterally in the Vsup and in the dorsomadial margin of the trigeminal principal nucleus adjacent to the Vsup. These findings have for the first time demonstrated features of the central processing of muscle sensation.

(COI: No)

P3-008

Analysis on the inter-areal axon projection in the mouse neocortex

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Cerebral neocortex integrates different sensory inputs with internal status to elicit an appropriate behavior. Direct neuronal connections between functional areas within a cerebral hemisphere should take an important part in this process. Long association fibers (LAFs) are the long-range connections between distant areas located in different cortical lobes and recent studies have reported that the LAFs are disturbed in the mental/developmental diseases like schizophrenia and autism spectrum disorders, suggesting the importance of LAFs in cognitive functions. However, the detailed axonal structure of long association neurons (LANs) that constitute the LAFs and how its final structure is established during cortical development are yet to be revealed. To study the structure and development of the LANs, we identified the mouse genes preferentially expressed in LANs compared to callosal neurons, which connect bilateral hemispheres via corpus callosum, by microarray analysis. We confirmed that some of the candidate genes obtained were indeed expressed in LANs in the primary somatosensory area (S1), by double labeling with in situ hybridization and retrograde tracing from primary motor cortex (M1) to the S1. Using the promoters of these LAN-specific genes and tissue clearing methods combined with deep brain imaging, we visualized the entire axon structure of individual neurons at different time point of the development.

(COI: No)

P3-009

Features of projecting neurons of the nucleus of the tractus solitarius

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The caudal nucleus of the tractus solitarius (cNTS) contains many neurons in the dorsal region (the subpostremal and dorsolaretal subnuclei) and the ventral region (the commissural and medial subnuclei) that innervate other brain regions, but information regarding their somal size and distribution remains incomplete. Here we labeled projection neurons in the cNTS with a retrograde tracer, the B subunit of cholera toxin (CTb), and studied their somal distribution and size in relation to their projection sites, including the parabrachial nucleus (PB), periventricular nucleus (PVN), central nucleus of the amygdala (CeA), and periaqueductal grey (PAG). Major findings include: 1) The PB projecting cNTS neurons were located in the subposremal region and the dorsomedial subnucleus. Their somal size was relatively small. 2) The PVN projecting cNTS neurons were preferentially localized around the tractus solirarius in the medial subnucleus. Their somal size was relatively large. 3) The CeA projecting cNTS neurons were preferentially localized just above the dorsal motor nucleus of the vagus nerve in the ventral region. Their somal size was medium. 4) The PAG projecting cNTS neurons were localized around the border between the medial and commissural nuclei in the ventral region. Their somal size was medium. Our findings, in combination with results of previous studies showing a spatial laminar segregation of neuronal populations: a dorsal group of high excitation and a ventral group of balanced excitation and inhibition, suggest that neuronal processing mechanisms in the cNTS might be different in relation to efferent projection system.

(COI: No)

P3-010

Gustatory pathways from the parabrachial nuclei to the ventral part of the caudate putamen via the caudal part of the intralaminar thalamic nuclei in rat brain

Iwai, Haruki; Kuramoto, Eriko; Yamanaka, Atsushi; Goto, Tetsuya (*Grad. Sch. Med. & Dent. Sci. Kagoshima Univ., Kagoshima, Japan*)

Background: The medial parabrachial nucleus (MPB) and external part of the medial parabrachial nucleus (MPBE) relaying gustatory information in the rat have been reported to project to the caudal part of the intralaminar thalamic nuclei. The intralaminar thalamic nuclei are known to project to the caudate putamen (CPu), however, it is unclear where the caudal part of the intralaminar thalamic nuclei projects within the CPu. The objective of this study was to determine morphologically their brain areas.
Methods: We visualized the parabrachio-thalamo-striatal pathways with anterograde and retrograde tracers.
Results: The MPB or MPBE projected to the ventrocentral part of the CPu via the caudal part of the oval paracentral thalamic nucleus; to the ventrolateral part of the CPu via the ventrolateral part of the parafascicular thalamic nucleus; to the ventromedial part of the CPu via the ventrocaudal part of the central medial thalamic nucleus, ventromedial part of the parafascicular thalamic nucleus, and retroreuniens area; and to the most ventral part of the CPu via the parvicellular part of the posteromedial ventral thalamic nucleus.
Conclusions: We demonstrate that the ventral part of the CPu receives projections from the caudal part of the intralaminar thalamic nuclei. Since the ventral part of the CPu has been reported to involve in food intake and jaw movement, our results suggest that gustatory information from the MPB and MPBE affects oral function within the ventral part of the CPu.

(COI: No)

P3-011

Analysis of calcitonin gene-related peptide (CGRP)-expressing neurons in the peripeduncular nucleus during lactation

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During lactation, suckling stimulus is conveyed to the hypothalamus via the brain stem and causes various behavioral and endocrinal changes, such as suppression of reproductive function. In the present study, we purposed the identification of neurons conveying suckling stimulus from the brain stem to the hypothalamus in lactating rats. We found significant increase in the expression of calcitonin gene-related peptide (CGRP) alpha in the brain stem in lactating rats using the quantitative RT-PCR analysis. CGRP-immunoreactive (ir) neural cell bodies were found in the peripeduncular nucleus of the mesencephalon (PP) and the fibers were found in the ventromedial nucleus of the hypothalamus (VMH) and caudal part of caudate-putamen (CPu) in lactating rats. The ratio of cFos-ir to the CGRP-ir neurons in the PP in suckling-stimulated mother rats was greater than that in non-stimulated control. After ipsilateral injection of biotinylated dextran amine (BDA; anterograde tracer) into the PP, BDA-labeled fibers were found in the CPu and VMH. Moreover, after injection of Fluoro-Gold (FG; retrograde tracer) into the CPu, FG-labeled cell bodies were found in the PP and positive for CGRP. On the other hand, FG injection into the VMH also caused labeling for several PP neurons but not positive for CGRP. These results suggested that CGRP neurons in the PP projecting to the CPu play a key role in conveying suckling stimulus to various brain regions during lactation.

(COI: No)

P3-012

Projection pattern of geniculocortical afferents in mouse visual cortex

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The visual information is received at the retina and carried to the visual cortex through the dorsal lateral geniculate nucleus (dLGN) of thalamus. In primates and carnivores, the dLGN contains mainly three types of neurons projecting to the cortex, which have distinct characteristics in physiological property, dendritic morphology and axonal projection pattern in the cortex. In mice, however, it has not been established yet whether there are multiple types of projection neuron in the dLGN. Although three types of projection neuron were reported in mouse dLGN based on their dendritic morphology (Krahe et al., 2011), it remains unknown whether they have different axonal projection patterns in the cortex. While the dLGNs of primates and carnivores are segregated into anatomically distinct layers which contain specific types of neurons, the dLGN of mice does not have such laminar organization. Thus, multiple types of neurons are intermingled in mouse dLGN, making it difficult to examine the projection patterns of a particular type of neurons with conventional neuronal tracers. We established a method to visualize the entire axonal and dendritic structures of a few neurons in dLGN using the *in vivo* local electroporation technique (Ohmura et al., 2014). Using this novel technique, we found that mouse dLGN neurons show several distinct patterns of axonal projection in the cortex. These results suggest that the dLGN of mouse contains multiple types of projection neurons which represent parallel visual pathways.

(COI: No)

P3-013

Functional architecture of the gloss selective regions in the monkey inferior temporal cortex

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We have previously reported that there exist neurons that selectively responded to specific range of gloss in the inferior temporal (IT) cortex of the monkey (Nishio et al., 2012), and these gloss selective neurons encode perceptual gloss parameters (Nishio et al., 2014). Gloss selective neurons were concentrated in a restricted region extending 2-3 mm in the lower bank of the superior temporal sulcus (STS) in the central IT cortex. To understand the cortical processing related to the generation of gloss selective neurons, in the present study, we injected a small amount of retrograde tracer (CTB Alexa 555) in the lower bank of STS where gloss selective neurons were clustered. We observed retrogradely labelled neurons were distributed in several regions posterior to the injection site including area V4 and the posterior IT cortex. Labelled neurons were most densely observed within the STS, in particular at the lip of the STS in the posterior IT cortex, but we also observed clustering of labeled cells in the lateral surface of the IT gyrus dorsal to the anterior end of the posterior middle temporal sulcus (PMTS). These regions seem to correspond to the regions where an image of shiny object evoked strong responses in our previous fMRI experiment (Okazawa et al., 2012). These regions may from a network that is specially related to the processing of gloss information in IT cortex.

(COI: No)

P3-014

Analyses on the candidate receptors for the axon collateralization of the developing corticospinal tract

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Axon collaterals are involved in the coordination of neural activities required for the higher brain function. During development, corticospinal tract extends interstitial axon collaterals from their main shaft toward the multiple targets. Several lines of evidence suggest that a diffusible collateral-inducing factor(s) is released from the target. However, the molecular entities and mechanisms that induce and elongate the axon collaterals are mostly unknown. We selected candidate ligands for the collateral induction over the corticospinal tracts by choosing the molecules that were strongly expressed in their targets, such as the pontine nuclei and the superior colliculi. In addition, with the aid of the public database, we finally listed 105 candidate receptors. To test whether these receptors are truly involved in the axon collateralization, individual receptor in the layer V neuron of the cortex was disrupted using the RNAi interference. So far, we identified several potential candidates that are involved in collateral formation: knockdown of 10 candidate receptors resulted in poor collateral formation to the pontine nuclei, whereas that of 4 candidate receptors enhanced collateral formation.

(COI: No)

P3-015

Roles of Runx1 in muscle innervation of mouse embryonic hypoglossal neurons

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Runx1, runt-related transcription factor, plays important roles in the cell type specification and axonal projections of the nociceptive dorsal root ganglion (DRG) neurons. In our previous study, we found that Runx1 was expressed in the ventrocaudal part of hypoglossal nucleus (nXII) at embryonic day (E) 17.5. In *Runx1*-deficient mice, areas immunoreactive for vesicular acetylcholine transporter (VAChT; a presynaptic marker of motor axon terminals) were reduced in the vertical and transverse tongue muscles, whereas those in the genioglossus (GG) muscle were increased, suggesting that some hypoglossal neurons switch their targets in the absence of Runx1. In the present study, to address this possibility, we examined hypoglossal neurons using retrograde labeling. Cholera toxin B subunit (CTB) was injected into the GG muscle of E17.5 embryos, and the localization of CTB-labeled motoneurons was examined. The distribution of CTB-labeled hypoglossal neurons in *Runx1*-deficient mice was similar to that of control mice, indicating that Runx1 deficiency did not alter axonal projections to the GG muscle. We also examined the expression of two markers of cranial motoneurons, c-Met and c-ret. Althouth Runx1 regulates c-Met or c-ret expression in DRG neurons, Runx1 deficiency did not change the expression of these motoneuron markers. Thus, it is unlikely that the altered axonal projection from nXII in *Runx1*-deficient mice is associated with c-Met or c-ret dysfunction.

(COI: No)

P3-016

Intrinsic projections of the retrosplenial cortex in the rabbit: Projections to dysgranular area 30

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The retrosplenial cortex (RS) is an essential structure for discriminative avoidance learning in the rabbit. RS consists of areas 29a, b, and c, and area 30, but the connectivity between these areas has not been studied yet. Here, we studied afferent projections to area 30 from other areas within RS, with the retrograde tracing method. Eleven male New Zealand White rabbits weighing 2.5-3.0 kg were used. Under anesthesia, a single iontophoretic injection of cholera toxin B subunit (CTB) was made in a various rostrocaudal level of area 30. After 7 days, the rabbits were perfused with a fixative, and their brains were cut into sections, which were treated immunohistochemically to visualize retrogradely labeled cells. Injection of CTB into part of rostral area 30 labeled cells over the rostral one-third of area 30 and in area 29c. Injection into the mid-rostrocaudal part labeled cells distributing over the middle two-thirds of area 30 along the rostrocaudal axis and in area 29b. Injection into part of caudal area 30 labeled cells over the caudal one-third of area 30 and in area 29b. These labeled cells occurred in layers 2-6. Contralateral labeled cells occurred in layers 2 and 5 and superficial layer 6 of areas 30 and 29b/29c at the rostrocaudal level of each injection site. These results suggests that the rostral and caudal parts of area 30 may function independently, and mid area 30 may integrate information from the rostral and caudal parts of RS.

(COI: No)

P3-017

Tyrosine hydroxylase (TH) immunoreactive fibers unsusceptible to the degeneration occurring in the zitter mutant rat originate from the dorsal tier of the substantia nigra compact part (SNC)

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The zitter rat is an autosomal recessive mutant rat derived from the Sprague-Dawley (SD) strain and these mutant rats show fine tremor and flaccid paresis progressing with aging as well as curled body hair and bent whiskers. The zitter mutant rat also exhibits the degeneration of the TH immunoreactive fibers in the striatum with aging. We reported previously that these mutant rats exhibited the region-specific vulnerability in the TH immunoreactivity with more severe in the dorsal striatum than in the ventral striatum (Ueda et al, Neuroscience, 2000). However little is known about why this region-specific vulnerability occur. To identify neurons projecting to the ventral striatum, we injected a retrograde tracer, fluorogold (FG) into the ventral striatum of normal rats and examined the location of FG labeled neurons and its neurochemical properties. We found that 1) many FG labeled neurons were present in a dorsal tier of the SNC but not in a ventral tier of the SNC and 2) all FG labeled neurons were TH immunoreactive neurons. These results suggest that TH immunoreactive fibers unsusceptible to the degeneration occurring in the zitter rat originate from the dorsal tier of the SNC and support our previous findings showing that the dopaminergic neurons in the ventral tier of the zitter mutant rat is more vulnerable than that in the dorsal tier (Ueda et al, Neurosci letters, 2005).

(COI: No)

P3-018

The local field potential in the forebrain by the optogenetic manipulation of serotonergic neurons in the raphe nucleus

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Serotonin (5-HT) is a neurotransmitter involved in a wide range of brain functions as a modulation of multiple type of behaviors such as appetitive, emotional, motor, cognitive and autonomic. Optogenetics is an elegant tool to control neurotransmitter release with millisecond precision and cell type-specific resolution. We previously succeeded in generating transgenic mice that expressed a light-sensitive channelrhodopsin-2 variant ChR2(C128S) in serotonergic neurons. The optogenetic stimulation of serotonergic neurons in the dorsal raphe nucleus (DRN) caused a robust increase of serotonin release in the medial prefrontal cortex (mPFC). Furthermore, the activation of DRN enhanced patience for a future reward when the animal is deciding whether to keep waiting or to abandon the wait (Miyazaki et al, 2014 Curr Biol.). The ventral hippocampus is known to be important targeted region of serotonin as well, because several types of serotonin receptors (Htrs) such as Htr1a, 2a, 2c, and 7 are highly expressed. However, it is unclear how serotonin modulates the activities of targeted neurons in these forebrain structures. To clarify the effect of the serotonergic modulation on the neuronal activities in the forebrain structures with high temporal and spatial precision, we recorded local field potential (LFP) in prefrontal cortex and ventral hippocampus by a sixteen-channel silicon probe. Our preliminary results showed that the activation of serotonergic neurons by optogenetics evoked LFP response in the mPFC and ventral hippocampus.

(COI: No)

P3-019

Differential innervation of the efferent nerves in the rat testis

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In the mammalian testes, autonomic efferent nerves are innervated via the superior and inferior spermatic nerves. These post-ganglionic neurons may be originated from the sympathetic chains, pre-vertebral ganglia and pelvic ganglia, however, the detailed distributions of these neurons are still unclear. To examine the distributions of testicular efferent neurons, retrograde tracer Fluorogold (FG) was injected into the testicular nerves in the rat. A microcapsule filled with 2% FG was inserted to the cut-end of the left testicular nerves. After 3 days, the autonomic ganglia and the brains were removed and serially frozen sections were made, then, FG-labeled cells were observed and counted in each ganglion. As the results, Labeled cells were distributed in the ipsilateral sympathetic ganglia (sympathetic chains: 74.8%; the prevertebral ganglia: 16.7%) and the contralateral ganglia (sympathetic chains: 8.7%). Only a few FG-labeled cells were found in the ipsilateral pelvic ganglia. No labeled cells were observed in the parasympathetic ganglia. Almost all FG-labeled cells were represented for tyrosine hydroxylase immunoreactivity. These results suggest that the neurons projecting to the testis via superior spermatic nerves may be all sympathetic and originated mostly from the neurons located in the ipsilateral sympathetic chains. Furthermore the distribution of these neurons may reflect the descensus of the testis and its vascular system during development.

(COI: No)

P3-020

Systematic analysis methods of in situ hybridization labeled cells

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It is meaningful to know more information about in situ hybridization (ISH) labeled cells, so we attempted to analyse those cells one more step. But owing to the tissue damage derived from ISH staining process, it was hard to utilize the same tissue following ISH for more analysis. Therefore, in order to avoid the influence of this tissue damage, we made some kinds of efforts. And we managed to perform this analysis. In consequence, we developed some techniques of analysis for ISH labeled cells. 1) Comparison of the distribution with the cells expressing other gene or protein : ISH double staining, ISH + immunohistochemistry (IHC). 2) Search for other expressing genes in those cells : laser microdissection of ISH labeled cells + single cell PCR. 3) Visualization of the morphology of those cells : DiI injection into ISH labeled cells. Combining these techniques, we analyzed ISH labeled cells systematically.

(COI: No)

P3-021

Large-volume optical reconstruction of brain tissue with a resolution of single synapses

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Branching pattern of dendrites and distributions of spine synapses along dendrites are basic information for structural analysis of neural circuits. Tissue sectioning techniques are usually applied for such analysis, but visualization of the entire dendritic arborization requires labor-intensive procedures, including acquisition, alignment, and reconstruction of a large number of sections. Possible deformation and destruction of structures by sectioning itself is an additional concern. CLARITY, a recently-developed technique of tissue processing, enables rapid access of exogenous antibodies, large volume imaging, and improvement of the optical property, which is suitable for the use of an objective lens with a high numerical aperture. This technique is suitable for wide range microscopic observation inside the tissue without sectioning. We report that the CLARITY technique can be applied to observe entire dendrite morphologies of cortical pyramidal neurons with a resolution of single spines. Additional immunohistochemistry of the sample enabled us to evaluate molecular compositions of each synapse and the extent of contact with glial processes. CLARITY technique applied to high-resolution imaging of dendrites should be useful in detecting morphological phenotypes in mouse models of neurological and psychiatric disorders.

(COI: No)

P3-022

Sema4D is involved in microglial polarization after cerebral ischemia

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Brain ischemia evokes microglial activation. There is increasing evidence that activated microglia are polarized to functional distinct phenotype: proinflammatory M1-like microglia and anti-inflammatory M2-like microglia. This phenomenon shows the importance of controlling microglial function in therapy of brain ischemia. Sema4D is a 150 KDa transmembrane and secreted-type semaphorin belonging to the classIV semaphorin subclass. Sema4D promotes some immune functions, such as activation of B-cells, dendritic cells and T-cells. Although microglia express Plexin B1 and CD72 which are receptors of Sema4D, the interactions of microglia and Sema4D remain unclear. Here, we show that influence of Sema4D on microglial phenotype after cerebral ischemia by permanent middle cerebral artery occlusion. Sema4D-deficiency inhibited microglial polarization to M1-like, whereas promoted to M2-like after cerebral ischemia. Although there was no change in the mRNA levels of polarization-related cytokines, ERK1/2 phosphorylation which is downstream of Plexin B1 was inhibited by Sema4D. These results suggest that Sema4D directly affects microglial polarization.

(COI: No)

P3-023

Interferon regulatory factor 7 participates in the M1-like microglial polarization switch

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Microglia are generally considered the immune cells of the central nervous system. Recent studies have demonstrated that under specific polarization conditions, microglia develop into two different phenotypes, termed M1-like and M2-like microglia. However, the phenotypic characteristics of M1-like- and M2-like-polarized microglia and the mechanisms that regulate polarization are largely unknown. In this study, we characterized LPS-treated M1-like and IL-4-treated M2-like microglia and investigated the mechanisms that regulate phenotypic switching. The addition of M2-like microglial conditioned medium (CM) to primary neurons resulted in an increase in neurite length compared with neurons treated with M1-like microglial CM, possibly because of the enhanced secretion of neurotrophic factors by M2-like microglia. M1-like microglia were morphologically characterized by larger soma, while M2-like microglia were characterized by long processes. M2-like microglia exhibited greater phagocytic capacity than M1-like microglia. These features switched in response to polarization cues. We found that expression of interferon regulatory factor 7 (IRF7) increased during the M2-like to M1-like switch in microglia in vitro and in vivo. Knockdown of IRF7 using siRNA suppressed the expression of M1 marker mRNA and reduced phosphorylation of STAT1. Our findings suggest that IRF7 signaling may play an important role in microglial polarization switching.

(COI: No)

P3-024

The change of Morphology and Distribution of Microglia in the postnatal developing mouse cerebellum

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Microglia have been considered as immune cells which are activated by pathological events, but recent analyses suggest that they also play crucial roles in postnatal development of the immature brain. However, distribution and morphology of microglia in the developing brain remain poorly understood especially in the cerebellum. In the present study, we morphologically examined developmental changes of microglia in the mouse cerebellum. Mice aged from postnatal day 5 (P5) to P60 were transcardially perfused and microglia were labeled by Iba1 antibody. Around P5, majority of microglia was distributed in white matter. The density of microglia in the white matter massively decreased until P13. In contrast, the density of microglia was stable and identical among the internal granular layer, the Purkinje cell layer and the molecular layer after P8. Taking increase in the cortical volume during postnatal development into account, these results suggest that microglia migrate from the white matter to the cortex from around P7. We found that morphology of microglia was also changed in parallel with the cortical migration. At P5, microglia in the white matter tended to have large bodies and poor branching processes. Those in the cortex had fine long processes and small somata after P13, which is similar to those in adult mice. Taken together, these results suggest that translocation and maturation of microglia massively proceed from around first postnatal week to P13 in the mouse cerebellum.

(COI: No)

P3-025

Immunohistochemical study of axonic satellite glial cells in rat DRG

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Initial segment of neuronal process of the dorsal root ganglion (DRG) neuron is covered by axonic satellite glial cells followed by myelinating Schwann cells (Pannese in 1960). However, after this report, there had been no report on axonic satellite glial cells. In the present study, we elucidated features of axonic satellite glial cells in 6 weeks old rats by immunohistochemistry and BrdU histochemistry. About 10 axonic satellite glial cells covered approximately 100 μm of initial segment of the neuronal process. Each of the glial cells covered one neuronal process. These glial cells locating near the neuronal cell body (proximal region) showed satellite glial cell markers, Kca2.3, and weak p75 immunoreactivity. Axonic satellite glial cells situated near myelianting Schwann cell (distal region) strongly showed p75 immunoreactivity, but not Kca2.3. Moreover, the glial cells in distal region were also immuno-positive for promyelinating Schwann cell marker, Oct-6. To examine their proliferation and differentiation, BrdU was injected and observed with time course. Two hours after BrdU administration, BrdU was observed in axonic satellite glial cells which are close to myelianting Schwann cells. Two weeks after administration, BrdU was detected in most proximal myelinating Schwann cells. These results suggest that axonic satellite glial cells are composed of some cell population, and that the glial cells locating in distal region are Schwann cell precursors.

(COI: No)

P3-026

Hedgehog Signaling Regulates the Morphogenesis of Schwann Cell in Specific Time Windows

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Although hedgehog (Hh) signaling is one of the key signaling for regulating the myelination in peripheral nerve system, detailed mechanism has not been elucidated. We previously reported that Hh signaling was received by Schwann cells (SCs) through the primary cilia, and facilitated the myelination. Importantly, the ratio of primary cilia-positive SCs gradually increased from the promyelinating phase to the initial stage of myelin sheath formation (Yoshimura and Takeda, 2012). These results indicate that the SCs autonomously determine the sensitive period of Hh signaling for myelin formation, and Hh signaling chiefly play a crucial role during the early stage of myelination. In this study, we determine the detailed function of Hh signaling in promyelinating stage of mouse Schwannoma cell line TR6Bc1. In promyelinating stage, SCs become quiescent and form the primary cilia. Furthermore, mature SCs migrate along axons and extend their processes. When Hh signaling was activated by Smo agonist, maturation and process formation were significantly facilitated. However, other steps, such as proliferation and migration, were not affected. These results demonstrate that Hh signaling is specifically received by SCs to form the bipolar morphology in promyelinating stage. To further establish the function of Hh signaling for morphological change in SCs, we are now trying to construct the Smoothened (Smo: effector of Hh signaling) knock out cells by CRISPR/Cas9 system.

(COI: No)

P3-027

Myelination at the peripheral-central transitional zone of developing chick vestibulocochlear nerves

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The eighth cranial nerve consists of the vestibular and cochlear nerves. Our previous study showed regional differences of myelination in the chick vestibular and cochlear nerves. In this study, an immunohistochemistry with antibodies specific to Schwann cell marker protein zero (P0), oligodendrocyte marker proteolipid protein (PLP) and myelin basic protein (MBP) were used to detail the myelination at the peripheral nervous system (PNS) and central nervous system (CNS) transitional zone of the vestibular and cochlear nerves in embryonic chicks. Embryos in 9-14 day eggs (E9-14) were prepared for the immunohistochemistry. In the vestibular nerve, the immunoreactivity of PLP and MBP was first observed in CNS at E10 and that of P0 and MBP in PNS at E11. In the cochlear nerve, the immunoreactivity of PLP and MBP was first observed in CNS at E10 and E11, respectively, and that of P0 and MBP in PNS at E13. And then, positive axons gradually increased. These observations suggest that the onset of the myelination in CNS is earlier than that in PNS of both the vestibular and cochlear nerves. Moreover, the myelination in the vestibular nerve should occur earlier than that in the cochlear nerve. It seems that the myelination of Schwann cells is preceded by that of oligodendrocytes and the development of the auditory function is by that of the vestibular function.

(COI: No)

P3-028

Characterization of Olig2-positive astrocytes in the normal adult forebrain

Tatsumi, Kouko; Okuda, Hiroaki; Morita, Shoko; Wanaka, Akio (*Nara Med. Univ., Nara, Japan*)

Olig2, a basic helix-loop-helix (bHLH) transcription factor, persists in the central nervous system from embryonic to adult stages. In the adult stage, nearly all Olig2 positive cells co-express NG2 proteoglycan, and constitute a subpopulation of oligodendrocyte precursors (OPCs). So-called "adult OPCs" have abilities to self-renew and to differentiate. Our genetic labeling study in the adult brain revealed that Olig2 positive cells generate NG2 glia (OPCs), oligodendrocytes and astrocytes (Tatsumi et al., 2008; Islam et al., 2009; Okuda et al., 2009). Recently we found a new subpopulation of Olig2-positive cell in the gray matter of the adult forebrain. They are post-mitotic and GFAP-positive, but do not express NG2 proteoglycan. These Olig2-positive astrocytes are distributed widely in the adult brain with clustering in the basal ganglionic nuclei such as the globus pallidus (GP) and the substantia nigra pars reticulata. Both of these nuclei receive inhibitory GABAergic signals from the striatum and the GP respectively, suggesting that Olig2-positive astrocytes extend their fine processes around the inhibitory synapses. Assuming the tripartite synapses theory, Olig2-positive astrocyte may contribute to inhibitory transmission in the adult forebrain.

(COI: No)

P3-029

Role of pro-oligodendroblast antigen in oligodendrocyte differentiation

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The pro-oligodendroblast antigen (POA) that reacts with the pro-oligodendroblast-specific antibody O4 has not been identified biochemically. The O4 also reacts with sulfatide (HSO3-3-galactosylceramide) at the mature OL stage, but sulfatide synthesis at the pro-oligodendroblast stage is uncertain. In the present study, we showed by imaging mass spectrometry that sulfatide existed in restricted regions of the ventricular zone of the spinal cord at embryonic 13.5 mouse, where pro-oligodendroblasts first appear. At this stage, short-chain sulfatide with 20 carbon fatty acids was predominant, while long-chain sulfatide with 24 carbon fatty acids was dominant in adult spinal cord. We examined OL differentiation in cerebroside sulfotransferase (Cst) -null mice that lack sulfatides. The number of immature OLs at embryonic 14.5 in Cst-null mice was lower than that in wild type cervical spinal cord. However, the population of the immature cells in Cst-null mice increased rapidly and became comparable with that in wild type mice at embryonic 16.5. Moreover, in primary OL culture from embryo, significant decreasing in the number of immature OLs was shown at 3 day in vitro in Cst-null mice and it recovered to a normal level at 5 day in vitro. Together, these results demonstrate that POA is the sulfatide species with short-chain fatty acids and regulates the early OL development.

(COI: No)

P3-030

Yokukansan ameliorates glucocorticoid receptor protein expression in oligodendrocytes of the corpus callosum after stress exposure

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Major depressive disorder is probably the oldest and still one of the most frequently diagnosed psychiatric illnesses. Major depressive disorder is one of the leading causes of disturbances in emotional, cognitive, autonomic, and endocrine functions, affecting nearly 7% of the population in Japan. According to the large amount of information on depressive diseases that has been accumulated during recent years, patients with major depressive disorder show an enhanced biologic stress-response mechanism, especially a hyperactive hypothalamic-pituitary-adrenal (HPA) axis and high levels of circulating cortisol. Although dysregulation of the HPA axis by chronic stress is indicative of major depressive disorder, the molecular mechanisms and functional changes in the brain underlying depression are largely unknown. Recently, we reported that stressed mice with elevated plasma levels of corticosterone exhibit morphological changes in the oligodendrocytes of nerve fiber bundles, such as those in the corpus callosum. However, little is known about the molecular mechanism of GR expression regulation in the oligodendrocytes after stress exposure. In this study, by using water-immersion and restraint stress as a stressor for mice, we attempted to elucidate the GR regulation mechanism in the oligodendrocytes and evaluate the effects of Yokukansan, a *Kampo* medicine, on GR protein level regulation.

(COI: No)

P3-031

Oligodendrogenesis of hippocampal axon fibers in the fornix of adult mouse

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Oligodendrocytes are generated at late development to form myelin sheath for proper signal transmission and neuronal survival, recently oligodendrocyte progenitor cells (OPCs) are shown to distribute more or less evenly throughout the adult brains in whole life. The present study showed that proliferation of OPCs in the fornix, main axonal pathways of hippocampal neurons, was regulated coordinately with that of neural stem/progenitor cells (NSPCs) in the hippocampus after antidepressant treatment. A peripheral inflammatory stimulation with lipopolysaccharide (LPS) attenuated proliferation of OPCs in the fornix and NSPCs in the hippocampus, but conversely induced robust and transient proliferation of microglia. A microglia inhibitor minocycline suppressed proliferation of OPCs and LPS-induced attenuation of OPC, indicating that microglia play a fundamental role in regulating both basal proliferation of OPCs under normal condition and attenuation of OPC proliferation under inflammatory condition. The administration of vascular endothelial growth factor signaling inhibitor suppressed basal proliferation of OPCs and LPS-induced proliferation of microglia. In conclusion, this study indicates that microglia play crucial function in controlling OPC proliferation in the fornix of adult brains.

(COI: No)

P3-032

Hedgehog signal modulates the release of gliotransmitters from astrocytes

Okuda, Hiroaki; Tatsumi, Kouko; Morita, Shioko; Wanaka, Akio (*Nara Med. Univ, Nara, Japan*)

Hedgehog (Hh) signaling pathway is conserved in a wide range of species from drosophila to human and plays a key role in regulating organogenesis. The sonic hedgehog (Shh), a member of Hh family, is an essential factor for central nervous system development. Shh stimulates differentiation of neural stem cells into motor neurons or interneuron in the neural tube and proliferation of immature cells in cerebellum and retina. Hh signaling molecules are also found in the adult brain, implying that Hh signaling functions in the mature nervous system. In the present study, focusing the function of Hh signaling in the adult mouse brain, we first examined expression of Hh signaling molecules in the adult mouse brain by in-situ hybridization. Patched 1, which is a receptor of Hh family members, was expressed in S100beta positive astrocytes and Shh mRNA was expressed in HuC/D-positive neurons in the adult mouse cerebellum. These results suggest that Hh is involved in neuron-glia interaction. We further confirmed that the Hh signal molecules were expressed in cultured cerebellar astrocytes using RT-PCR. We next examined whether or not recombinant Shh N-terminal (rShh-N) activates Hh signaling pathway and regulates astrocytic functions in vitro. rShh-N treatment induced D-serine release and inhibition of Hh signaling pathway led to decrease in glutamate and ATP release from cultured cerebellar astrocytes. These findings suggested that Hh signal pathway modulates release of gliotransmitters and is related to neuro-glial interactions in the adult mouse brain.

(COI: No)

P3-033

The roles of fatty acid desaturase on the differentiation of cultured neural stem cells

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Polyunsaturated fatty acids (PUFAs), especially docosahexaenoic acid (DHA) and arachidonic acid, are essential for the growth and functional development of the brain. In postmortem human brain tissues of Alzheimers disease, depressive disorder, and schizophrenia, decreasing PUFA levels and decreasing mRNA levels of enzymes for PUFA synthesis were observed. Moreover, there is a failure in cell proliferation and differentiation of neural stem cells (NSCs) in these diseases. We hypothesized that enhancement of PUFA synthesis in the brain is an important target to prevent and treat these diseases. The present study examined roles of PUFA synthetic enzymes on the differentiation of the cultured rat fetal NSCs. Addition of 1% fetal calf serum (FCS) increased the percentage of GFAP (an astrocyte marker) -positive cells. Addition of B27, a medium supplement that increases neuronal survival in primary CNS cultures, increased the percentage of Tuj-1 (a neuronal marker)-positive cells. FCS and B27 treatment increased the mRNA levels of stearoyl-CoA desaturase, delta-5 and delta-6 desaturases, and fatty acid elongase-5 via sterol regulatory element-binding protein (SREBP) 1c transcriptional activation. SREBP1c is known as a main regulator of PUFA synthesis. These results suggest that activation of PUFA synthesis is involved in promoting the differentiation of NSCs.

(COI: No)

P3-034

Protective effects of a novel nucleic acid analogue (COA-Cl) against oxidative damage in PC12 cells

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COA-Cl is a synthesized nucleoside analogue with the molecular weight of 284. We previously reported that it has angiogenic potency both *in vitro* and *in vivo*. We also found that COA-Cl promoted the synthesis and secretion of VEGF, the most robust pro-angiogenic growth factor in human fibroblast.

In this study, we investigated the neuroprotective effects of COA-Cl on H_2O_2-induced apoptosis in rat pheochromocytoma (PC12) cells. H_2O_2 (0-200 μM, 24 h) increased LDH release from PC12 with decrease in cell viability. However, treatment with COA-Cl (100-200 μM) significantly reduced LDH release and attenuated the decrease in cell viability dose dependently. In addition, immunoblot analysis showed that COA-Cl inhibited H_2O_2 -induced apoptosis by increasing the Bcl-2/Bax ratio. We also examined the ability of COA-Cl against oxidative damage caused by a neurotoxin 6-hydoroxydopamine (6-OHDA), which has been widely used to generate the experimental model of Parkinsons disease. COA-Cl showed the similar protective effects against the 6-OHDA induced oxidative stress to those against H_2O_2. Collectively, COA-Cl might be considered to be a promising neuroprotective agent against oxidative damage.

(COI: No)

P3-035

Prosaposin overexpression after kainic acid-induced neurotoxicity

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Excessive glutamate release plays a pivotal role in numerous neuropathological disorders, such as ischemia or seizure. We aimed to investigate whether intrinsic prosaposin (PS), a neuroprotective factor when supplied exogenously in vivo or in vitro, is up-regulated after the excitotoxicity induced by kainic acid (KA), a glutamate analog. In this study, PS immunoreactivity and its mRNA expression in the hippocampal and cortical neurons showed significant increases on day 3 after KA injection, and high PS levels were maintained after 3 weeks. The increase in PS, but not saposins, detected by immunoblot analysis suggests that the increase in PS-like immunoreactivity after KA injection was due to an increase in PS as a neurotrophic factor to improve neuronal survival. Furthermore, several neurons with slender nuclei inside/outside of the pyramidal layer showed more intense PS mRNA expression than other pyramidal neurons. These neurons were shown to be GABAergic interneurons in the extra- and intra-pyramidal layers. Several large neurons in the V layer of the cerebral cortex and the choroid showed very intense PS mRNA expression after KA injection. This study indicates that inhibitory interneurons as well as stimulated hippocampal pyramidal and cortical neurons synthesize PS for neural survival, and the choroid plexus is highly activated to synthesize PS, which may prevent excitotoxic neural damage. This study demonstrates axonal transport and increased production of neurotrophic factor PS after KA injection.

(COI: No)

P3-036

Abnormal lysosomes accumulating in Cathepsin D-deficient mouse neurons are targeted for selective autophagy through p62 and NBR1

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Cathepsin D (CD)-deficient mice show a new form of lysosomal accumulation disease with a phenotype resembling neuronal ceroid lipofuscinosis (NCL). In neurons deficient in CD, abnormal lysosomes called granular osmiophilic deposits (GRODs) and autophagosomes accumulated in the perikaryal regions. The present study shows that a part of GRODs were incorporated into double or multimembranous autophagosomes, while such autophagosomes with GRODs were not observed in neurons of mouse brains doubly deficient in CD and Atg7, in which autophagy cannot be executed. In these single knockout mice of CD and even in double knockout mice of CD and Atg7, p62 and NBR1, adaptor proteins of selective autophagy were co-localized with ubiquitin on the limiting membranes of GRODs by immunoelectron microscopy using the cryo-thin section-immunogold method. These results suggest that p62 and NBR1, together with ubiquitin, are involved in selective autophagy of GRODs in CD-deficient mice.

(COI: No)

P3-037

Quantitative analysis of development and aging of genital corpuscles in glans penis of the rat

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The development of genital corpuscle (GC) in human has previously been described by using classical histological methods such as methylene blue staining or silver impregnation. However, other species, including rats, have not been examined and a quantitative study of GC development is also lacking. This study report a quantitative evaluation of the development and aging of GCs in the rat glans penis using protein gene product 9.5 (PGP9.5) immunoreactivity as a neuronal marker. In addition, neural elements in the glans penis were studied by immunohistochemical staining for calcitonin gene-related peptide (CGRP), substance P (SP), vasoactive intestinal polypeptide (VIP), and neuropeptide Y (NPY). GCs were identified as corpuscular endings consisting of highly branched and coiled axons with many varicosities, which were immunoreactive for PGP9.5. GCs were also immunoreactive for CGRP and SP, but not for VIP and NPY. The results revealed that densities and sizes of GCs in the rat develop postnatally, reach the peak of development after puberty, and continue to exist until old age, in contrast to prenatal and early postnatal development of other sensory receptors of glabrous skin. These results suggest possibility that GCs develops under influence of sex hormone.

(COI: No)

P3-038

Translational machinery and protein synthesis in growth cones of rat dorsal root ganglion neurons; atomic force microscopic and fluorescence microscopic analysis

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Although the concept of local translation in neurons is widely accepted, there is a debate about whether axonal translation occurs. Herein, we analyzed the presence of ribosomal proteins in the growth cones of rat dorsal root ganglion (DRG) neurons, by immunofluorescence analysis. Actual protein synthesis was monitored by puromycin technology. Structural analysis was performed using atomic force microscopy (AFM). DRG neurons were prepared from embryonic rats and dissociated using trypsin. DRG neurons were resuspended in culture medium and plated onto dishes. They were maintained in DMEM containing CPT-cAMP to facilitate axon elongation and growth cone formation. Brain-derived neurotrophic factors were applied to induce translational activation under the presence of puromycin. After AFM observation, specimens were labeled with Alexa 488 phalloidin for actin filament staining, followed by anti-ribosomal protein P0/P1/P2 antibody. Immunofluorescence images revealed that actin filaments were distributed in the peripheral region and in the filopodia. The positive regions of ribosomal protein P0/P1/P2 were closely related to the distribution of actin filaments. AFM images showed that high regions of DRG tended to be rich in actin filaments and ribosomal protein P0/P1/P2, compared with low regions of DRG. These results are discussed in relation to locally-synthesized proteins and are related to the three-dimensional structure of DRG.

(COI: No)

P3-039

Expression of nitric oxide synthase (NOS) isoforms after peripheral nerve transection in mice

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The distribution and function of nitric oxide synthase (NOS) in various tissues and organs have been investigated. There are 3 isoforms of NOS: nNOS present in nerve cells, eNOS present in vascular endothelial cells, and iNOS synthesizing NO in response to cytokine stimulation. In this study, to clarify the action of NOS in neurodegeneration and nerve regeneration after transection of the sciatic nerve in mice, we immunohistochemically investigated NOS expression. The right sciatic nerve was cut in mice. The nerve was excised with the surrounding tissue and frozen sections were prepared. The sections were immunostained following the standard method and observed under a light microscope. Strong NOS positivity was detected in the nerve fiber stump on the central side after transection. Inflammatory cells infiltrating around the stumps on the central and peripheral sides were positive for iNOS on day 1, but the positivity level decreased after day 7. eNOS was positive in blood vessels on the central and peripheral sides of the stump from day 1. nNOS-positive nerve fibers were noted mainly on the central side of the stump, and then extended toward the peripheral side, suggesting that NO is involved in the nerve regeneration process.

(COI: No)

P3-040

Localization of VIP immunoreactive and NPY immunoreactive neurons in the rat submandibrar ganglion

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Introduction: The submandibrar ganglion(SMG) have been considered to be involved in parasympathetic nervous system. In many physiological studies have been reported on account of SMG was not just relay nucleus, but also may have more complicated function. This study was intended to examine distribution of NPY immunoreactive and VIP immunoreactive neurons in SMG with a confocal laser microscope. Furthermore, ultrastructure of SMG was investigated in conventional optical and electron microscopes.
Material and Methods: Animals were fixed, SMG were removed, and frozen sections were cut at 20 micrometer thickness. Sections were treated for double-immunohistochemical demonstration of VIP and NPY and examined with a confocal laser microscope.
Results and Discussion: Although most of SMG neuron showed NPY immunoreactivity, neurons around of the hilum were negative. The ganglion neuron around of the hilum showed VIP immunoreactivity, and on the other hand NPY immunoreactive neurons were not obseved. No morphological difference was demonstrated between the neuron around the hilum and in the neuron of any other parts by electron microscope. Most of SMG neurons showed NPY immunoreactivity and VIP immunoreactive neuron were localized around the hilum. Conclusion was that SMG was related with the superior cervical ganglion.

(COI: No)

P3-041

Generation and characterization of a transgenic rat line expressing Venus under control of the gastrin-releasing peptide promoter

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We previously demonstrated that the sexually dimorphic gastrin-releasing peptide (GRP) system in the lumbosacral spinal cord mediates male sexual function, and this spinal system is developed and regulated by an androgen dependent manner. In parallel, it has been reported that the somatosensory GRP system in the spinal cord contributes to the regulation of itch specific transmission independently of the pain transmission without a sexual dimorphism. The purpose of this study is to establish the animal model that is able to efficiently analyze two different spinal GRP systems controlling male sexual function and itch sensation *in vivo*. Therefore, we generated the GRP-Venus transgenic (Tg) rat expressing Venus under control of the GRP promoter. We first observed the Venus fluorescence in the lumbosacral spinal cord of GRP-Venus Tg rats. Using immunohistochemistry, we also found that most GRP neurons in the lumbosacral spinal cord co-expressed Venus fluorescence. In addition, in GRP-Venus Tg females, a long-term androgen treatment significantly increased the number of Venus-positive neurons in the lumbosacral spinal cord. RT-PCR analysis further confirmed the expression of Venus mRNA both in the lumbosacral spinal cord and spinal ganglion. Thus, GRP-Venus Tg rat model appears to be a powerful tool for analyzing spinal GRP systems controlling male sexual function and itch sensation.

(COI: No)

P3-042

Expression of Trk-fused gene protein in the motor neurons of the rat corticospinal tract

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The *TRK*-fused gene (*TFG* in human, *Tfg* in rat) has originally found in cancer tissues as a part of fusion-oncoprotein in anaplastic lymphoma and mixoid chondrosarcoma. In the normal cells, studies were shown that TFG involves in ER-golgi protein secretion and the NF-kB pathway signaling. Although TFG mutations were found neurodegenerative diseases affecting motor and sensory functions in hereditary motor and sensory neuropathy with proximal dominant involvement (HMSNP), hereditary spastic paraplegias, and Charcot-Marie-Tooth disease type II (CMT2), a role of TFG in the nervous system remains unclear. We have previously produced an antibody against rat TFG and used it to localize TFG to selected neurons in specific regions in the rat brainstem. In the present study, we investigate in TFG immunoreactivity in the motor neurons in the brainstem and spinal cord of the rat corticospinal tract. We have identified the TFG-positive neurons in the parts of cranial motor neurons in the rat brainstem and motor neurons in the ventral horn of the spinal cord. Our TFG expression data provides better understanding of TFG in the motor system.

(COI: No)

P3-043

Masticatory muscle inflammation prolongs MAPK activation in the brainstem

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Object: The mechanism of myofascial pain syndrome (MPS) is little known. The aim of our study is to elucidate the mechanism of MPS, so we examined the effect of MAPK in the trigeminal subnucleus caudalis (Vc) activation induced by a noxious stimulation of the left masseter (LM).
Methods: The LM of Sprague Dawley rats (male, 250g, n=60) was stimulated as follows: 1) L-S6 (experimental) group; The rat's LM was injected with lipopolysaccharide 2 μg/kg (100 μl) on the 1st day of the experiment. On day 2, the same site was injected with 6% sodium chloride solution (S6, 100 μl, 5 times per 90 min). 2) S-S (control)group: The rat's LM was injected with normal saline (S, 100 μl) on the 1st day of the experiment. On day 2, the same site was injected with S (100 μl). Rats were allowed to survive for 1 day, 7 days or 14 days after the last injection. The masseter s and brainstems were dissected and cut with a cryostat (at 30 μm thickness). These specimens were investigated with anti-bradykinin receptor B2 (BKRB2, masseter) or anti-p-p38 MAPK (brainstem) enzyme labeled antibody method. The specimens were observed and evaluated using a light microscope mounted with a 3CCD digital camera system.
Results: The BKRB2-immunoreactive (IR) cells both groups were observed until 7 days after stimulation. In the experimental group, the p-p38 MAPK-IR cells were particularly observed in the Vc until 14 days after stimulation. However, the p-p38 MAPK-IR cells in the control group were little existed until 3 days.
Conclusion: The MAPK activation expression is activated by chronic pain.
(COI: No)

P3-044

Prosaposin and its receptors in the cerebellum after kainic acid injection

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Prosaposin (PSAP), a highly conserved glycoprotein, is a precursor of saposins A-D. Accumulating documents suggest PSAP to be a neurotrophic factor in vivo and in vitro that induces differentiation and prevents death in a variety of neuronal cells through the active region within the saposin C domain. Recently, GPR37 and GPR37L1 were recognized as PSAP receptors. In this work, we explored the variation in expression of PSAP and its receptors in Purkinje cells from the cerebellum by immunohistochemistry using rats injected with kainic acid (KA). The data show that PSAP was expressed in the cytoplasm of Purkinje cells and was markedly enhanced on days 3, 7, and 14 following KA treatment. Meanwhile, the expression of GPR37L1 was increased on days 1 and 3 but not on day 7 or 14 compared with rats receiving normal saline. In contrast, the expression of GPR37 was significantly diminished on days 7 and 14, in contrast to the expression pattern of GPR37L1. These findings indicate that PSAP protects Purkinje cells from damage induced by KA with the aid of its receptors, GPR37L1 and GPR37, and that these receptors have diverse effects during this process.
(COI: No)

P3-045

Deletion of *Crmp4* results in altered morphology and physiology in the olfactory bulb

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Collapsin response mediator protein 4 (CRMP4) is suggested to be involved in neuronal development. Since previous reports showing roles of CRMP4 were mainly performed *in vitro*, information about roles of CRMP4 *in vivo* is insufficient. And no physiological phenotypes in *Crmp4*-knockout (KO) mice have been reported, making it difficult to elucidate *in vivo* roles of CRMP4. Our previous study showing strong expression of *Crmp4* mRNA in the olfactory bulb (OB) from postnatal day (PD) 0 to PD7 suggested important roles of CRMP4 in OB development. Here, we aimed to explore phenotypes of *Crmp4*-KO pups by examining morphology and physiology of the OB. In morphological studies on the OB, *Crmp4*-KO pups had longer apical dendrites of mitral cells and thicker external plexiform layer whose main constituents are mitral cells' apical dendrites, compared to those in WTs. With physiological analyses, we found that *Crmp4*-KO pups exhibited impaired olfactory discrimination ability by measuring ultrasonic vocalizations emitted from pups. Activity-dependent c-Fos expression revealed that *Crmp4*-KO pups exhibited hyperactivities in the OB. In addition, mRNA expressions of not GABA receptors but glutamate receptors of AMPA type were increased in *Crmp4*-KO pups than WTs, suggesting that enhanced excitatory circuits contribute to their hyperactivity phenotype. Our data indicate that CRMP4 is involved in the morphological as well as physiological development of the OB.
(COI: No)

P3-046

Immunohistochemical analysis of opsin 5, an ultraviolet-absorbing photopigment, in chicken and mouse neural tissues

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Opsin 5 (Opn5) is one of the recently identified opsin groups that is responsible for nonvisual photoreception in animals. We previously showed that a chicken homolog of mammalian Opn5 (Opn5m) and mouse Opn5m are Gi-coupled ultraviolet sensors. We demonstrated that mouse Opn5m evolved to be a more specialized photosensor by losing one of the characteristics of bistable pigments, direct binding of all-trans-retinal, which is acquired by a single amino acid replacement. Thus, chicken and mouse Opn5m have a different molecular property. To know whether there might be different physiological functions of Opn5m between mouse and chicken, here we analyzed the expression patterns of Opn5m in chicken and mouse neural tissues. We found that, like chicken Opn5m, mouse Opn5m was localized to a small subset of cells in the ganglion cell layer and inner nuclear layer of the retina. On the other hand, the mouse Opn5m was expressed in the preopic area of the hypothalamus, while chicken Opn5m is expressed in the posterior hypothalamus, specifically paraventricular organ, which is known to be photosensitive in invertebrates.
(COI: No)

P3-047

Newly-identified sexually dimorphic gene expressions in the mouse medial preoptic area of the hypothalamus

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The medial preoptic area (MPOA) in the anterior hypothalamus has crucial roles for the sexually dimorphic behaviors and physiological regulations. The steroid hormone receptors are abundantly expressed in the MPOA, and such receptor expression must be involved in the behavioral difference between sexes. It is also well known that the some MPOA subregions show the sexual difference in their volume, the neuron density and/or the fiber density (called sexually dimorphic nucleus, SDN). The regulatory role of SDN for some behaviors or physiology, however, has not been identified because of the poor understandings in their anatomical aspects, especially in their gene expression. In this study, we examined the sexually dimorphic gene expression in the mouse MPOA. Several candidate genes which expressed in specific MPOA subregions were picked up through the database search in the Allen Brain Atlas. The cDNAs of candidate genes were subcloned, and transcribed to synthesize riboprobes. We performed *in situ* hybridization on the adult mouse brain sections to elucidate their sexually dimorphic expression. As a result, at least five genes showed sexually dimorphic expressions in the MPOA, and three of them were newly identified molecular markers for the SDN. We also performed the double-labeling study of c-Fos and mRNA, and will discuss the possible role for the SDN on the sexually dimorphic behaviors.
(COI: No)

P3-048

Relationship between cFos- and nitric oxide synthase-immunoreactivity in neurons in the rat subfornical organ after intraperitoneal injection of hypertonic saline

Kawano, Hitoshi; Masuko, Sadahiko (*Saga Univ., Saga, Japan*)

It is well known that the subfornical organ (SFO) is an osmosensor and administration of hypertonic saline induces elevation of neuronal activity in the SFO. It is essential to clarify the characteristics of such neurons. There are many nitric oxide synthase (NOS)-immunoreactive neurons in the SFO, thus the present study was designed to reveal the relationship between cFos-immunoreactivity (a marker for elevated neuronal activity) neurons after administration of hypertonic saline and NOS-immunoreactivity in SFO. Male SD rats were intraperitoneally injected 5 ml of hypertonic (5.265%) or isotonic (0.9%, control) saline (n=3, each), anesthetized 5 min later, and perfused with 4% paraformaldehyde. cFos-immunoreactivity was found in 3.8% and 1.1% of NOS-immunoreactive neurons in the SFO of the hypertonic and isotonic saline injected rats, respectively, although the ratio was not significantly different. This finding suggests that a certain population of NOS-immunoreactive neurons in the SFO is responsible for elevation of neuronal activity in the SFO in response to application of hypertonic saline.
(COI: No)

P3-049

The expression of the oxytocin-monomeric red fluorescent protein 1 fusion gene in the hypothalamus and spinal cord of acute nociceptive model rats

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Oxytocin (OXT) is a well-known neurohypophysial hormone that is synthesized in the paraventricular (PVN) and the supraoptic nuclei (SON) of the hypothalums. Several lines of evidence have suggested that OXT plays an important role in pain modulation and analgesia. However, little is known about the neuronal spinal networks responsible for OXT effects. The present study examined the effects of acute nociceptive stress on the expression of the OXT-monomeric red fluorescent protein 1 (mRFP1) fusion gene in the hypothalamus and spinal cord of the transgenic rats. As the acute nociceptive model, OXT-mRFP1 transgenic rats were subcutaneously injected with formalin at the bilateral hindpaws. We observed mRFP1 fluorescence in the PVN, the SON, and the dorsal horn in the spinal cord after formalin injection. The expressions of the mRFP1, and the OXT gene in the hypothalamus were also measured by in situ hybridization histochemistry. We revealed that mRFP1 and OXT mRNA levels in the PVN and the SON and the mRFP1 fluorescence in the dorsal horn were significantly increased at 2 hour after formalin injection compared with controls. We have assessed whether increased hypothalamic OXT may associate with spinal pathway and influence mechanical nociceptive threshold.

(COI: No)

P3-050

The effects of hormonal fluctuation during pregnancy and postpartum on the expression of estrogen receptor α and neuronal morphology in the amygdala

Matsuo, Seiki[1,2]; Matsuda, Kenichi[1]; Nishimura, Yusuke[1]; Kishimoto, Anju[1]; Yoneda, Mari[1]; Yokoo, Shiho[1]; Kitawaki, Jo[2]; Kawata, Mitsuhiro[1] (*[1]Dept Anat Neurobiol, Kyoto Pref Univ Med, Kyoto, Japan; [2]Dept Obst Gynecol, Kyoto Pref Univ Med, Kyoto, Japan*)

During pregnancy from the first trimester to a few months after delivery, some of the women suffer from depression. Amygdala is one of the central regions which regulate emotion. We investigate the effect of hormonal fluctuation during pregnancy and postpartum on the expression of estrogen receptor α and neuronal morphology in the amygdala. We used virgin female Wistar rats [gestational day 15 (G15), 20 (G20), 4 days after delivery (P4), and normal estrous (E)] to perform immunohistochemistry and the Rapid Golgi Stain. Rats were perfused with paraformaldehyde. And the brains were sectioned, incubated with anti-ER α and treated with diaminobenzidine. At P4, the number of ER α immunoreactive cells in the central amygdala was decreased compared to G15 and G20 ($p<0.05$). The rat brains were stained by using the Rapid Golgi Stain protocol. We selected pyramidal neurons in the amygdala. And the dendritic spines were counted along the first branch of the apical dendrite and spine density was determined by the number of the spines in 10 μm. At P4, the number of mushroom type spine density in the amygdala was decreased compared to G15, G20 and estrous ($p<0.05$). These results suggest that the perinatal dynamic changes of the estradiol concentrations may affect to the morphological changes in the amygdala through the response with ER α , resulting in emotional instability.

(COI: No)

P3-051

Effects of cutaneous stroking on the responses to immobilized stress of serotonin releasein the central nucleus of the amygdala in rats

Takagi, Noriaki[1]; Tokunaga, Ryota[1]; Shimoju, Rie[1,2]; Shibata, Hideshi[4]; Maruyama, Hitoshi[1]; Kurosawa, Mieko[1,2,3] (*[1]Physical Ther, Health & Welfare Sci, Intl Univ Grad Sch Health & Welfare, Tochigi, Japan; [2]Center Med. Sci., Intl. Univ. Health & Welfare, Otawara, Japan; [3]Dept. Pharm. Sci., Intl. Univ. Health & Welfare, Otawara, Japan; [4]Lab. Veterinary Anatomy, School of Veterinary Medicine, Tokyo University of Agriculture*)

The present study aimed to examine the effects of cutaneous stroking on responses of 5-HT release in the CeA to immobilization in rats. A coaxial microdialysis probe was stereotaxically implanted in the CeA and perfused with modified Ringer's solution at a speed of 1 μl/min. The dialysate output from the probe over consecutive periods of 10 min was manually injected into the HPLC and the amount of 5HT was measured with an electro-chemical detector. Rats were placed for 20 min in a handmade restraint box made of paper clay, and fixed all four limbs to a board using strings and adhesive tape. Stroking stimulation was applied manually to the back during immobilization period. The 5HT release in the CeA significantly increased during 20 min-immobilization period. On the other hand, the 5-HT release showed no significant changes in response to the immobilization when stroking was applied simultaneously. These results demonstrate that cutaneous stroking can eliminate the responses of 5-HT release to immobilization stress. Since 5-HT in the CeA is known to cause fear and anxiety behaviors, present results suggest that tactile stimulation of the skin dampen these emotions induced by immobilization stress.

(COI: No)

P3-052

Responses of serotonin release in the central nucleus of the amygdala to cutaneous stroking of rats are mediated via type1 corticotropin releasing factor receptors in the dorsal raphe nucleus

Tokunaga, Ryota[1]; Takagi, Noriaki[1]; Shimoju, Rie[2,4]; Shibata, Hideshi[5]; Yamaguchi, Rie[5]; Kurosawa, Mieko[2,3] (*[1]Physical Ther., Health & Welfare Sci., Intl. Univ. Grad. Sch. Health & Welfare, Tochigi, Japan.; [2]Center Med. Sci., Intl. Univ. Health & Welfare, Otawara, Japan; [3]Dept. Pharm. Sci., Intl. Univ. Health & Welfare, Otawara, Japan; [4]Dept. Physical Ther., Intl. Univ. Health & Welfare, Otawara, Japan; [5]Lab. Veterinary Anatomy, School of Veterinary Medicine, Tokyo University of Agriculture and Technology*)

We have found that responses of serotonin (5-HT) release in the central nucleus of the amygdala (CeA) to cutaneous stroking were abolished after administration of a non-selective corticotropin releasing factor (CRF) receptor antagonist, α-helical-CRF (9-41), into the dorsal raphe nucleus (dRN) in anesthetized rats. In the present study we examined the contribution of both type 1- and type 2- CRF receptors in the dRN to the responses of 5-HT release. A coaxial microdialysis probe was stereotaxically implanted in the CeA and perfused with modified Ringer's solution at a speed of 1 μl/min in anesthetized rats. Stroking stimulation was applied to the back for 10 min. After vehicle injection into the dRN, stroking stimulation decreased the 5HT release. The responses of 5-HT to stroking were abolished after injection of antalarmin, a type 1 CRF receptor antagonist, while those were not influenced by antisauvagine-30 (ASV-30), a type 2 CRF receptor antagonist. These results suggest that responses of 5HT release in the CeA to cutaneous stroking are mediated via the type 1 CRF receptor in the dRN.

(COI: No)

P3-053

Immunohistochemical analysis and behavioral tests for mdx52 mice, a model for DMD

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Duchenne muscular dystrophy (DMD) is a severe X-linked degenerative disorder of the muscle. This disorder is caused by mutations of dystrophin gene. Besides the muscle, full-lengh dystrophin protein, Dp427, is expressed in the brain. Dystrophin is expressed in neurons of the cerebral cortex, cerebellum, hippocampal CA1-CA3 regions and amygdala basolateral nucleus (BLA). Dp427 is an actin-binding scaffold protein selectively localizes in the postsynaptic membrane of GABAergic synapses. Previous studies suggest that a lack of Dp427 induces reduction of the number of $GABA_A$ receptor α2 subunit clusters and failure of maturation of GABAergic synapse in *mdx* mice, a model for human DMD. Besides Dp427, isoforms from *DMD* gene, such as Dp140 and Dp71, are express in the brain. Recent analysis of mutation in *DMD* gene indicates two deletion 'hot spots', which are the regions exons 2 to 20 and exons 45 to 50. Mutation in the former induces a deficit in the expression of Dp427 only, but that in the latter induces deficits in the expression of both Dp427 and Dp140. Previous clinical studies suggest that the mutation in *DMD* gene that induces deletion of Dp140 in addition to Dp427 correlate with cognitive deficits and psychiatric symptoms. However, other studies do not find significant effects of Dp140 deletion in central symptom. The effects of Dp427-deletion in non-intellectual abnormality are not understood. In this study, we examined immunohistochemical staining of GABAA receptors in the BLA and several behavioral tests for *mdx52* mice, which lack both Dp427 and Dp140 because of deletion in the exon 52 in *DMD* gene.

(COI: No)

P3-054

Altered localization of FoxO1 in neuron by methamphetamine

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The mammalian forkhead box O (FoxO) is a family of transcription factors consisting of FoxO1, FoxO3a, FoxO4 and FoxO6. FoxO1 is expressed in liver, fat, pancreas β cells, brain and so on. It has been reported that FoxO1 is involved in various physiological functions, such as apoptosis, cell division and glucose metabolism. FoxO1 is downstream of PI3K/ Akt in the insulin signaling pathway. FoxO1 shifts into the cytoplasm and its transcriptional activity is decreased by phosphorylation by Akt. FoxO1 is abundantly expressed in the striatum and remains to be incompletely understood about the physiological function in the striatum. The striatum receives the modification by dopamine nerves. Then, we investigated whether FoxO1 in the striatum is regulated by dopamine by administering dopamine-related drugs to ICR mice and conducting immunostaining. FoxO1 translocated to the nuclei from the cytoplasm by the administration of metamphetamine, which increases dopamine concentration in the synapse cleft. Metamphetamine is known to also promote the release of catecholamines such as noradrenaline, but the nuclear translocation of FoxO1 by metamphetamine was inhibited by pre-treatment of each SCH23390, antagonist for D1 dopamine receptors and Haloperidol, antagonist for D2 dopamine receptors. Moreover, co-administration of each A68930, agonist for D1 receptors and Qunipirole, agonist for D2 receptors was required for the nuclear translocation of FoxO1. From the above findings it was suggested that dopamine is involved in the transcriptional regulation of striatal FoxO1.

(COI: No)

P3-055

Ferulic acid decreases serotonin metabolism of the striatum in vivo with no tocxicity to dopaminergic neurons

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Ferulic acid (FA) is a phytochemical compound naturally presents in several plants and foods, having antioxidant effect. It is recently known that FA exerts beneficial action in depressive-like behaviors and it interacts to monoamine neurotransmitter such as dopamine (DA), norepinephrine and serotonin (5-HT). To know FA effects on DAergic neurons, we first investigate the toxicity to cultured DAergic neurons. DAergic neurons were prepared from embryolic mesencephalon of Wistar rat and cultured in Neurobasal A + B27 at a density of 1.0 x 105 cells/cm2 for 5 days, followed by staining to tyrosine hydroxylase (TH). There was no change of TH-positive cell numbers by FA treatment (0-30 ug/ml). Furthermore, FA did not show neuroprotective effect of DAergic neurons against 6-hydroxyDA oxidative stress. We next investigated the effects of FA on monoamine metabolism in the striatum using in vivo microdialysis-HPLC. A tendency of decrease of HVA level as shown just after FA treatment. FA caused in decrease of 5-HT metabolite (5-HT and 5-HIAA), indicating that FA might interact serotonin synthetases (tryptophan hydoxylasearomatic or L-amino acid decarboxylase) in vivo. Data suggest that FA changes monoamine levels without toxicity to DA neurons.

(COI: No)

P3-056

GABAergic neurons are present as a cluster in the A11 region of rat brain

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A11 dopaminergic (DA) neurons are the only DA neurons that innervate the spinal cord and dysfunction of A11 DA system may cause restless legs syndrome. Based on recent findings, the DA neuron-enriched regions such as A8, A9, and A10 regions are composed of not only DA neurons but also GABAergic and glutamatergic neurons. Moreover, these non-DA neurons in the A10 region regulate neuronal activities of the DA neurons in the A10 region. However, little is known about neuronal composition of the A11 region. In this study, to determine whether or not the A11 region contain GABAergic or glutamatergic neurons, we performed DIG in situ hybridization for non-DA neurons and examined the distributions of the GABAergic and the glutamatergic neurons throughout the A11 region. Interestingly, we detected GABAergic neurons as a cluster in the middle of the A11 region and this cluster was located adjacent to a TH cluster, but not overlapped completely with the TH cluster. In contrast to the GABAergic neurons, the glutamatergic neurons were sparsely distributed in this region. These results suggest that 1) A11 region contain not only DA neurons but also GABAergic neurons and glutamatergic neurons as previously reported in the A8-A10 regions. 2) In the middle of the A11 region, GABAergic neurons are present as clusters adjacent to the TH clusters. These GABAergic neurons may regulate the activity of the DA neurons that project into the spinal cord.

(COI: No)

P3-057

Neuronal activations in midbrain regions after peripheral administration of peptide YY in mice: an effect of postingestive consequences

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Peptide YY (PYY), one of anorectic gut hormones, is released from gastrointestinal tract after meal and reduces subsequent food intake. Several studies showed that peripheral administration of PYY activates various brain regions including the nucleus of solitary tract, arcuate nucleus, dorsal striatum, nucleus accumbens (NAc), central nucleus of the amygdala and ventral tegmental area (VTA) in rodents. From these results we can assume that neural activations by PYY in several brain regions are involved in postingestive consequences such as reduction of hunger drive and/or visceral discomfort. To test this assumption, we explored neural activity in extensive midbrain regions using c-fos immunohistochemistry. Seventy to ninety minutes after an intraperitoneal administration of PYY, mice were perfused with 4% paraformaldehyde and brains were removed. Fos expressions were detected with immunohistochemical staining. Fos-immunopositive cells were obviously found in the VTA, rostromedial tegmental nucleus (RMTg) and reticulotegmental pontine nucleus. Furthermore, prominent Fos expression was found in the periaqueductal gray (PAG) and dorsal raphe (DR). These results suggest that neural activations by PYY in the ventral (VTA and RMTg) and dorsal (PAG and DR) midbrain regions contribute to positive and/or negative postingestive consequences after meal.

(COI: No)

P3-058

Moxibustion activates brain reward system of rats

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Moxibustion, one category of the Oriental medicine, originated in ancient China and developed in modern Japan. As a folk remedy, it is believed to be effective for keeping health and for the treatment of various chronic illness. In contrast to acupuncture, for which scientific evidences have accumulated very recently, nothing have been reported on the effect of moxibustion on the body. As the first step of its exploration, its effect on the brain was attempted in order to throw light on the physiological effect of moxibustion.

Rats treated with moxibustion were examined by brain microdialysis to measure secretion of dopamine, by conditioned place preference test (CPP) with unbiased method to know whether moxibustion acted as reward, and by immunohistochemical and quantative-PCR analysis of the brain tissues to measure the expression of c-fos as a marker for the activation of neuron.

Moxibustion treatment induced dopamine secretion in the nucleus accumbens, dorsal striatum and medial prefrontal cortex where the expression of c-fos was detected. Animals showed a preference to moxibustion in CPP.

These results suggested that moxibustion acted as a reward, and reactions of the dopamine reward system supported these physiological phenomena. This could be the first evidence on the effect of moxibustion on the body and be the fundamental basis for further clarifying the mechanism of the oriental medicine and development of its new therapy.

(COI: No)

P3-059

Chronological Changes of Prosaposin in the Dentate Gyrus after Birth

Matsuda, Seiji[1]; Morishita, Midori[1]; Nabeka, Hiroaki[1]; Shimokawa, Tetsuya[1]; Doihara, Takuya[1]; Yamamiya, Kimiko[1]; Kobayashi, Naoto[2]; Hamada, Fumihiko[3] ([1]*Grad. Sch. Med., Ehime Univ., Ehime, Japan;* [2]*Med Education C, Grad. Sch. Med., Ehime Univ., Ehime, Japan;* [3]*Anat, Ohita Med Univ., Ufu, Japan*)

Chronological Changes of Prosaposin in the Dentate Gyrus after Birth. Seiji Matsuda, Midori Morishita, Hiroaki Nabeka, Tetsuya Shimokawa, Takuya Doihara, Kimiko Yamamiya, Naoto Kobayashi*, Fumihiko Hamada**Anat Embryol, Education C*, Ehime Grad Med. Anat, Oita Med Univ**Prosaposin (PS), a highly conserved glycoprotein, is a precursor of saposins A-D. Many reports suggest PS to be a neurotrophic factor that induces differentiation in a variety of neuronal cells. This study investigated changes in PS in the dentate gyrus of young rats using double immunohistochemistry with antibodies to PS, PSA-NCAM, and NeuN. PS immunoreactivity was intense in the dentate gyrus at postnatal day 3 (P3) and P7, but decreased gradually after P14. In the dentate gyrus at P28, immature PSA-NCAM-positive neurons localized exclusively in the subgranular zone where neurons were PS -negative, whereas mature Neu-N-positive neurons were positive for PS. Laser microscopy images at higher magnification were examined for PS immunoreactivity in the nuclei and cytoplasm at P1, P7, P14 and P21. In situ hybridization assays showed that PS in the adult dentate gyrus is dominantly a secreted type of PS (Pro+9). These results imply that PS secreted from mature neurons stimulates proliferation and maturation of immature neurons in the dentate gyrus.

(COI: No)

P3-060

Immunohistochemical study on the substructures of the mouse subiculum

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Subiculum (Sub) is the major output part of the hippocampal formation. It receives afferents mainly from the CA1 region and sends efferents to many cortical and subcortical areas. Tracer experiments have revealed the topographies of these projections inside the Sub, but the cytoarchitecture of the Sub is not fully understood. So we explored the substructures of the Sub immunohistochemically using antibodies against several different substances. In line with previous studies, calbindin (CB)-immunoreactivity showed the border between the proximal and distal parts of the Sub, with the former being more intensely labelled than the latter. Both the CA1/Sub border and the Sub/presubiculum (PreS) border could also be determined by CB-immunolabelling. The sharp border between the Sub and PreS was further represented by immunolabeling for glutamate receptor 1. The above three borders among CA1/proximal Sub/distal Sub/PreS, which were delineated by CB-immunolabeling, matched the borders identifiable with vesicular glutamate transporter 2 (VGluT2)-immunolabelling. Moreover, a specific subregion in the proximal Sub was newly found by VGluT2-immunolabeling. This subregion was occupied by accumulation of large VGluT2-positive boutons that surrounded somata and proximal dendrites of VGluT2-negative neurons in the intermediate depth of the subicular cell layer. This subregion was also characterized by higher densities of parvalbumin-positive and SMI-32-positive neurons than the surrounding regions and was observable at the ventral part of the hippocampus but disappeared more dorsally.

(COI: No)

P3-061

A novel function of CPEB1 mRNA 3'UTR in dendrites

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The cytoplasmic polyadenylation element-binding protein 1(CPEB1) is a mRNA-specific translational control factor that has two RNA-recognition motifs and two zinc-finger motifs, and inhibits translation of CPE-containing target mRNAs by recruiting specific eIF4E-binding proteins, such as neuroguidin in neurons. Upon glutamatergic stimulation, CPEB1 is phosphorylated by either Aurora kinaseA or CaMk2 α, resulting in enhanced mRNA polyadenylation and local translation in post-synaptic regions. In this study, we focused on the mechanism of CPEB1 mRNA regulation, including RNA trafficking and activity-dependent local translation in dendrites. To investigate whether CPEB1 mRNA resides in dendrites, we visualized CPEB1 mRNA dynamics using the MS2-GFP system in living hippocampal neurons and found that the 3'UTR of CPEB1 mRNA was transported to distal dendrites and co-localized with either Staufen or CPEB1 itself. Furthermore, addition of CPEB1 3'UTR to GFP resulted in robust reduction in the GFP expression level compared to GFP alone although there was little down-regulation of its mRNA in hippocampal neurons. The precise mechanisms that regulate the translational repression of CPEB1 in dendrites have not yet been identified, but these results suggest that the CPEB1 might be controlled by other translational repressor(s) or CPEB1 itself, which enables the regulation of CPE-containing mRNAs in response to various neural stimuli.

(COI: No)

P3-062

Visual experience regulates MeCP2 expression in the dLGN in developing mice

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In the dorsal lateral geniculate nucleus (dLGN), retinogeniculate synapses develop through three phases. The first phase is synapse formation (P0-P10), and the 2nd is synaptic elimination and strengthening (P10-P20). These processes do not require visual experience. The 3rd is the visual experience-dependent synaptic maintenance phase (P20-), because visual deprivation for one week after P20 induces remodeling of retinogeniculate fibers. The Methyl CpG binding protein 2 (MeCP2) is reported to be important for visual experience-dependent maintenance of retinogeniculate synapses. However, the MeCP2 expression pattern in the dLGN throughout the three developmental phases has been unclear. MeCP2 immunopositive glutamatergic neurons in the dLGN were very few at P10 (11.1%), and then dramatically increased after P20 (>83.6%). Almost all GABAergic neurons were immunopositive for MeCP2 throughout the developmental phases (>98.3%). Interestingly, dark rearing from P21 for 10 days decreased MeCP2 expression only in glutamatergic neurons in the dLGN. These results raise the possibility that the MeCP2 expression level in glutamatergic neurons in the dLGN is regulated in visual experience-dependent manner during the synapse maintenance phase.

(COI: No)

P3-063

Molecular heterogeneity of perineuronal nets in the thalamic reticular nucleus

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The perineuronal net (PNN) was first described by Camillo Golgi in 1882 as a reticular structure that enwrapped the soma and dendrites of neurons. Seminal histochemical works have shown that PNNs are closely associated with parvalbumin-positive (PV+) GABAergic inhibitory neurons. Although PNNs and PV+ neurons play a critical role in regulation of neural plasticity in the developing visual cortex, the function of PNNs in other brain regions remains largely unclear. To address this issue, here we examined the molecular characteristics and topographic distribution of PNNs in the thalamic reticular nucleus (TRN), the gateway of thalamocortical pathways. The component of PNNs was characterized by using Wisteria floribunda agglutinin (WFA) and antibodies against proteoglycans. Considering the connections with cortical regions, the TRN was divided into three distinct sectors: the rostral sector is related to motor cortex and limbic areas, the intermediate sector is associated with somatosensory cortex, and the caudal sector has connections with visual and auditory cortices. In mature mice, WFA+ PNNs were seen in all sectors of the TRN. Interestingly, the labeling was more intense in the rostral and caudal sectors than in the intermediate sector. Aggrecan+ PNNs were most prominent in the intermediate sector, and they were rarely found in the caudal sector. Versican+ PNNs were seen in the dorsal part of the rostral sector only. PV+ neurons were seen in all sectors of the TRN. Our findings suggest that the molecular heterogeneity of PNNs in the TRN may be involved in regulation of topographically organized thalamocortical pathways.

(COI: No)

P3-064

Pin1 gene deficient mice impaired spatial cognitive function and exhibited frontotemporal lobar atrophy

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Pin1 is a ubiquitous peptidyl-prolyl cis/trans isomerase (PPIase) and has been shown to be necessary for cell growth and apoptosis. While pin1 deficit was suggested to contribute to Alzheimer's disease, the relation between behavior and pin1 has not been understood in detail. Pin1-/- and wild-type mice were obtained as a littermate and examined behavior test battery 9 to 24 month old. The test was Y-maze, open-field, object recognition and Morris water maze to examine the recognition, learning and memory function. After the test, the animals were measured T2-weight MRI images in brain and were sacrificed to collect brain samples followed by 4% PFA fixation. The brain was cut into 40-μm serial sections, and were stained by 1% thioflavin-S to detect β-amyloid. Pin1-/- mice showed significant difference with wild-type in object recognition and Morris water maze. Brain weight and volume by MRI did not show any different in both mice. However, the size in coronal plane at bregma -0.5 mm and more anterior showed a decrease in Pin1-/- mice. The mice also increased thioflavin-S reactions consisted with the piriform cortex. These results suggest that Pin1 deficit could impair entorhinal cortex including piriform cortex and result in spatial recognition impairment.

(COI: No)

P3-065

Excitatory and inhibitory inputs to vasoactive intestinal polypeptide-expressing neurons in the mouse primary somatosensory cortex

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GABAergic interneurons in the mouse neocortex have been classified into three subgroups based on gene expression: (1) parvalbumin-containing (PV+) neurons; (2) somatostatin-immunoreactive (SOM+) neurons; (3) 5HT3a receptor-expressing neurons, including vasoactive intestinal polypeptide-expressing (VIP+) neurons and the others. In the present study, we conducted morphological examination on the excitatory and inhibitory inputs to VIP+ neurons in layer 2/3 of the primary somatosensory cortex. The somata and dendrites of VIP+ neurons were visualized by using VIP-Cre knock-in mice and adeno-associated viral vectors (AAV2/1). The injected brain sections were then triple-immunostained for GFP, presynaptic markers and postsynaptic markers. The apposed presynaptic punctae on GFP-positive membrane were considered as putative input sites only when the postsynaptic markers existed on the apposed points. After reconstruction of somata and dendrites of VIP+ neurons in layer 2/3, the excitatory and inhibitory inputs to VIP+ neurons were counted. Both cortico- and thalamocortical excitatory inputs were frequently observed on the distal portions of dendrites of VIP+ neurons. On the other hand, each input of subgroups in GABAergic interneurons varied from proximal to distal; the inputs from PV+ neurons were frequently observed on the cell bodies and proximal dendrites of VIP+ neurons, whereas those of SOM+ neurons and VIP+ ones were mainly found on the distal dendrites.

(COI: No)

P3-066

Freely-moving mice exhibit emotional sweating on their soles in response to stress stimulus and during sleep from onset -A finding by the use of sweating-aided electrocardiogram floor sensor-

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Non-primate mammals have eccrine sweat grands only on their soles of paws. The sweating from the soles is considered to be prerequisite to improve the friction that is needed when they do hunt, fight, escape or climb up a tree. Such sweating is defined as emotional sweating, which is mediated by sympathetic activity enhancement. We have been investigating an electrocardiogram (ECG) recording of freely-moving mice using a floor sensor, which is made of a plate with multiple stripes of gold-plated electrode on the surface and designed to detect ECG when at least two paws touch different electrodes separately. However, actual ECG-detection rate of the sensor was very low because of the high electrode-skin contact impedance due to dried up soles, so that ECG appeared only when mice have sweat on their soles. In fact, ECG appeared immediately after hitting a cage with a stick, suggesting that the stress stimulus caused sweating by sympathetic activation. Interestingly, ECG also appeared during the period of Quiet Sleep in contrast to Quiet Waking state without ECG appearance. The heart rate immediately after the stress stimulus and 2-min after the onset of quiet sleep was 773 ± 21 (n = 8) and 374 ± 70 b/m (n = 5), respectively. The sweating during quiet sleep seemed not due to sympathetic activation because the heart rate was lower than the intrinsic heart rate (478 ± 36 b/m, n = 7). Further study is needed to clarify the unclear mechanism of the sweating during sleep on soles of paws in mice.

(COI: No)

P3-067

Oxidative stress in the lateral/ventrolateral periaqueductal gray does not play a role in evoking abnormal fear bradycardia in rats with heart failure

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We previously reported that 1) bradycardia in response to fear in rats was parasympathetically mediated by activation of lateral/ventrolateral periaqueductal gray (l/vlPAG), and that 2) fear bradycardia was enhanced in rats with heart failure (HF) compared to that in healthy controls. Mechanisms underlying abnormal fear bradycardia in HF have been unknown. In the present study, we attempted to determine if oxidative stress developing in the l/vlPAG of rats with HF contributes to enhancement of fear bradycardia. HF was induced in rats after myocardial infarction by coronary artery ligation. Superoxide generation in the l/vlPAG, as evaluated by dihydroethidium staining, was enhanced in rats with HF [fractional shortening (FS) < 25%, N=5] compared with that in sham-operated healthy rats (FS > 40%, N=5). In another set of conscious HF rats (N=11), five-min exposure of white noise sound (90 dB), which induced freezing behavior (an index of fear), evoked bradycardia response [-55 ± 10 beats per min (bpm) vs. baseline, mean ± SE]. Bilateral microinjection into the l/vlPAG of Tiron (200 mM, 100 nl), which has a superoxide scavenging activity, did not modulate the fear bradycardia (-60 ± 12 bpm). The present data did not support the concept that oxidative stress in the l/vlPAG plays a role in enhancing fear bradycardia in HF.

(COI: No)

P3-068

Expression of hemoglobin in presympathetic neurons of rat hypothalamic paraventricular nucleus with bombesin-induced activation

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A hemoglobin a-chain-derived peptide RVD-hemopressin is reported as a ligand for cannabinoid CB1 receptors. We previously showed that RVD-hemopressin inhibited centrally administered bombesin (a stress-related peptide)-induced secretion of adrenal catecholamines (adrenaline and noradrenaline) in the rat, suggesting an inhibitory role of brain cannabinoid system in the sympatho-adrenomedullary outflow. In this study, presympathetic spinally projecting neurons of the rat hypothalamic paraventricular nucleus (PVN), which have been shown to regulate the adrenal response, were labeled with a fluorescent retrograde tracer. Then, we examined the immunoreactivity of hemoglobin a-chain or RVD-hemopressin with neuronal activation marker Fos in the fluorescently labeled presympathetic neurons following the intracerebroventricular administration of bombesin. The bombesin induced Fos immunoreactivity in presympathetic PVN neurons with hemoglobin a-chain immunoreactivity. RVD-hemopressin-like immunoreactivity was also detected in presympathetic neurons of the PVN. These findings implicate the involvement of hemoglobin a-chain and RVD-hemopressin in modulating the sympatho-adrenomedullary outflow.

(COI: No)

P3-069

Hypothalamic mechanisms of autonomic nerve regulation by GLP-1

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In the present study, we examined effects of intracerebroventricular (ICV) injection of GLP-1 on autonomic nerve outflows in anesthetized mice, and found that GLP-1 dose-dependently increased sympathetic nerve activity to the kidney, and that activated renal nerve outflow was inhibited by pre-treatment of Exendin-3 (9-39), suggesting that sympathetic regulation by hypothalamic GLP-1 is mediated with GLP-1 receptor. In addition, sympathetic nerve activities to the white adipose tissue and the liver were stimulated by ICV injection of GLP-1. To clear hypothalamic intracellular mechanism, we investigated effects of ICV injection of GLP-1 on MAP kinase, PI3 kinase and PKA signaling in the hypothalamus, and found that GLP increased phosphorylation levels of PKA substrate, not ERK1/2 and Akt in mice. In addition, ICV injection of KT5720, PKA inhibitor, suppressed renal sympathetic response to GLP-1. Using rats, we found that c-Fos induction in the hypothalamic PVN was increased by ICV injection of GLP-1. Thus, these lines of evidence let us suggested that hypothalamic GLP-1 might act to PVN through the receptor-mediated intracellular PKA signaling and regulate autonomic nervous system for homeostasis.

(COI: No)

P3-070

The submandibular salivary secretory responses elicited by the activation of the non-NMDA and NMDA receptors in the superior salivatory nucleus neurons

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The parasympathetic preganglionic cells in the superior salivatory nucleus (SSN) receive inputs from sensory nerves as well as many of CNS nuclei. We investigated whether activation of the non-NMDA and NMDA receptors in the SSN neurons, performed by the microinjection of non-NMDA receptor agonist (AMPA, 0.1mM, 50nl, pH7.4, Sigma) and NMDA receptor agonist (NMDA, 0.1mM, 50nl, pH7.4, Sigma) into SSN region, elicit submandibular salivary secretory pressure responses in urethane-chloralose anesthetized rats. The submandibular salivary secretory pressure responses were elicited by microinjection of AMPA. Mean total volumes of the AMPA-induced saliva was 23.8 mg. The salivary secretory pressure responses was induced at mean latency of 23.6 seconds and lasted for mean time of 535 seconds. The average of the time constant in initial secretory pressure response was 34.4 seconds, and that of initial pressure increasing rates was 7.1 mmHg/s. The submandibular salivary secretory pressure responses were elicited by microinjection of NMDA. Mean total volumes of the NMDA-induced saliva was 12.4 mg. The salivary secretory pressure responses was induced at mean latency of 23.0 seconds and lasted for mean time of 190 seconds. The average of the time constant in initial secretory pressure response was 28.1 seconds, and that of initial pressure increasing rates was 6.8 mmHg/s. In conclusion, activation of the non-NMDA and NMDA receptors in the SSN neurons elicits submandibular salivary secretion.

(COI: No)

P3-071

Expression of c-Fos in the hypothalamus and the cardiovascular response during stress in Parkinson's disease model rats

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Parkinson's disease (PD) is a neurodegenerative disease that evoked by lack of the dopamine neurons in the substantia nigra of the midbrain. It is known that PD patients have an autonomic dysfunction, such as orthostatic hypotension. However, the mechanism of the autonomic dysfunction is still unclear. Recently, it has been reported that orexin neurons in the hypothalamus are reduced in the PD patients. In addition, the orexin neurons are localized at the hypothalamus, especially in perifornical area (PeF) and around dorsomedial nucleus (DMN). The PeF and DMN play an important role on the autonomic response to stress. Therefore, we hypothesized that function of the PeF and DMN against stress metamorphoses in the PD model. To verify this hypothesis, we stained c-Fos protein, a marker of neuronal activation, expressed by air-puff stress in the PD rats. The PD rat was developed by injecting 6-hydroxdopamine into the medial forebrain budle. After establishment of the PD model, the air-puff stress was made to the PD rat. In sham-operated rats, the air-puff stress caused pressor and tachycardic responses during the air-puff stress. However, increase in HR during the stress reduced in the PD rat. Expression of c-Fos protein in the DMN was similar level with the sham rat. In contrast, c-Fos positive-neurons in the PeF were significantly suppressed in the PD rat. The results suggest that cardiac dysfunction may occur in the PD model and the dysfunction is related to poorly-reactive neurons in the PeF against the stress.

(COI: No)

P3-072

Arterial blood pressure response to L-homocysteine microinjected in the rat ventrolateral medulla autonomic areas

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Elevated plasma L-homocysteine concentration is related to cardiovascular and neurological diseases. Arterial blood pressure (ABP) is regulated by the brain autonomic nuclei including the rostral ventrolateral medulla (RVLM) and caudal VLM (CVLM). However, the cardiovascular actions of L-homocysteine in those brain nuclei are unknown. ABP and heart rate (HR) are modulated by the homologue L-cysteine stimulation in the RVLM and CVLM of rats, via ionotropic NMDA and non-NMDA glutamate receptors. Therefore, the present study examined 1) if microinjected L-homocysteine influences ABP and HR in the RVLM and CVLM, and 2) if the action is mediated by ionotropic glutamate receptors. Microinjected L-homocysteine increased ABP and HR in the RVLM and decreased in the CVLM, the same as L-glutamate did, in urethane anesthetized paralyzed male Wistar rats. Prior microinjection of MK801 for NMDA receptor blockade, but not CNQX for non-NMDA receptor blockade, abolished responses in both brain nuclei. Results indicate central action of L-homocysteine via NMDA receptors of the autonomic brain nuclei. High concentration of L-homocysteine would stimulate NMDA receptors in the RVLM and CVLM cardiovascular neurons to influence cardiovascular regulation.

(COI: No)

P3-073

Activation of 5-hydroxytryptamine-1A receptors suppresses tachycardia evoked from the dorsomedial hypothalamus

Sato, Fumitaka; Nagaoka, Yuya; Horiuchi, Jouji (*Toyo University, Saitama, Japan*)

The psychological stress such as air-jet stress causes pressor response and tachycardia. The stress-induced response is mediated via the dorsomedial nucleus in the hypothalamus (DMH), so called "defense area". In addition, activation of serotonin 5-hydroxytryptamine-1A (5-HT1A) receptors in the central nervous system suppress the stress-induced autonomic response, though the central pathway of the response is still unclear. In this study, we investigated that effect of microinjection of 5-HT1A receptor agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) into the DMH on the cardiovasular response evoked by disinhibition (activation) of the DMH. Microinjection of bicuculline (BIC), GABAa receptor antagonist, in the DMH caused significant increases in blood pressure (BP), heart rate (HR) and renal sympathetic nerve activity (RNA) and these increases gradually returned to the pre-BIC injection level (30-40min). At approximately 10 min after the BIC injection, the all parameters plateaued at the peak, and then microinjection of 8-OH-DPAT was made into the same site of the BIC injection. Soon after the 8-OH-DPAT injection, HR increase elicited by the DMH activation started quicker reduction compare to the control BIC response without 8-OH-DPAT injection. In contrast, the 8-OH-DPAT in the DMH did not affect the pressor and tachycardic responses to the DMH activation. The results indicate that activation of 5-HT1A receptors located in the DMH inhibits the tachycardia evoked by stimulation of neurons in the DMH.

(COI: No)

P3-074

This poster presentation was withdrawn.

P3-075

Comparison of skin sympathetic nerve activities evoked by visual discrimination tasks

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We hypothesized that the skin sympathetic nerve activity (SSNA) contained sudomotor activity is evoked by voluntary movement to prevent slipping. We reported that SSNA bursts evoked by go trials of muscle contraction were related to MRCPs (2013). While sudomotor activity (SSNA followed by SSR: sympathetic skin response) is also known to be evoked by cognitive processing. We could not clarify the relationship between SSNA bursts with go trials and cognition. SSNA bursts were evoked by not only go trials but also no go trials. In this study, we compared SSNA bursts evoked by visual go and no go stimuli to investigate the difference of them. We recorded SSNA from the tibial nerve by microneurography, with corresponding sympathetic skin response (SSR). Electromyogram (EMG) was recorded from dorsal interossei muscles. To reveal cortical cognitive processing of visual cue (go or no go stimuli), electroencephalogram (EEG) on the scalp was recorded and averaged. Event related potential (ERP) was classified by the occurrence of SSR. The average amplitude of SSNA bursts of go trials was larger than that with no go trials. The average onset latency of SSNA bursts of go trials was longer than those of no go trials. In no go trials, ERPs classified by SSR showed the different pattern from ERPs classified by no SSR. These results suggest that SSNA bursts of visual no go trials may be related to cognitive information processing.

(COI: No)

P3-076

Tonic influence of corticotropin releasing factor on arterial pressure, heart rate, and serotonin release in the central nucleus of the amygdala in rats

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In addition to the well-known hormonal action of stimulating ACTH and adrenal cortical hormone secretion, corticotrophin-releasing factor (CRF) acts as a neuronal transmitter. For example, CRF neuron evokes fear and anxiety behavior and sympathetic activation such as increases in arterial pressure (AP) and heart rate (HR). However, tonic influence of the CRF neuron on these functions is not known. We therefore investigated the tonic influence of CRF on the AP and HR, and serotonin (5HT) release in the central nucleus of the amygdala (CeA), which causes fear and anxiety behavior, using of α -helical CRF(9-41), a non-selective CRF receptor antagonist. α -helical CRF(9-41) was i.c.v. administered in the anesthetized rats. AP was recorded continuously from the right carotid artery, and HR was calculated from the arterial pulse wave. 5HT release was measured with use of microdialysis technique and HPLC. Administration of α -helical CRF(9-41) significantly decreased AP and 5HT release in the CeA, but not HR. These results indicate that brain CRF contribute to stimulates arterial pressure and 5HT release tonically.

(COI: No)

P3-077

Muscarinic receptor-mediated excitation of rat intracardiac ganglion neurons

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Modulation of membrane excitability of rat parasympathetic intracardiac ganglion neurons by muscarinic receptors was studied using perforated patch recording configuration. Activation of muscarinic receptors by oxotremorine-M (OxoM) depolarized the membrane, accompanied with repetitive action potentials. OxoM evoked an inward current under voltage-clamp conditions at a holding potential of -60 mV. Removal of extracellular Ca^{2+} markedly increased the inward currents. The OxoM-induced current in the absence of extracellular Ca^{2+} was fully inhibited by removal of extracellular Na^{+}, indicating the contribution of non-selective cation channels. The OxoM-induced current was antagonized by muscarinic antagonists with following rank of affinity: 4-DAMP > pirenzepine > methoctramine, suggesting that M_3 receptors have a dominant role. The OxoM-induced current was inhibited by U73122, a phospholipase C (PLC) inhibitor. The membrane-permeable IP_3 receptor blocker xestospongin-C also inhibited the OxoM response. Furthermore, pretreatment with thapsigargin and BAPTA-AM inhibited the OxoM-induced current, while KN-62, a blocker of Ca^{2+}/calmodulin-dependent protein kinase II, had no effect. These results suggest that the activation mechanism involves PLC pathway, release of Ca^{2+} from intracellular Ca^{2+} stores and calmodulin. The cation channels activated by muscarinic receptors may play an important role in neuronal membrane depolarization in rat intracardiac ganglion neurons.

(COI: No)

P3-078

Functional mapping of visceral sympathetic nerve activity and skeletal muscle blood flow in the hypothalamus of the rat

Nagaoka, Yuya; Sato, Humitaka; Horiuchi, Jouji (*Toyo Univ., Saitama, Japan*)

It is known that there are two different types of behavioral response to stress. One is an active stress reaction known by "fight or flight", and another is a passive response known by "freezing behavior". Both reactions are mediated via the hypothalamus and accompany with the autonomic change. These active/passive reactions, which are recognized to "defense reaction", cause increases in blood pressure (BP), heart rate (HR), and skeletal muscle blood flow (SMF), and reduce visceral blood flow as a result of sympathetic activation. In the present study, we made functional mapping in the hypothalamus, especially in the dorsomedial nucleus and the marginal area (DMH), with measuring the SMF in the left hind leg and the renal sympathetic nerve activity (RNA) in the anesthetized rat. To activate neurons in the DMH, 15nl of DL-homocysteic acid (DLH, 50mM) was injected. As previously reported, the DLH microinjection into the DMH caused increases in BP, HR and RNA and the functional localizations of neurons was observed in the DMH. We also found the areas where increased or decreased the SMF at the left hind leg within the DMH. These opposite responses to the SMF were independent upon the responses to BP, HR and RNA. Therefore, we conclude that there are at least two populations of neurons to constrict or dilate skeletal vascular bed in the DMH and that the neuronal populations in the DMH may play important roles to make redistribution of the blood flow sympathetically during the stress condition.

(COI: No)

P3-079

Expression of c-Fos in the midbrain and the cardiovascular reaction during social defeat stress in rats

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Psychological stress caused by an interpersonal problem is involved not only the development of cardiovascular disorders but also depression and panic attack with excessive autonomic reaction. The stress reaction is elicited by neurons in the hypothalamus in mammals. It is still unknown, however, that the midbrain participates in the stress induced-autonomic response in mammals. In the present study, we investigated the distributions of expression of c-Fos (a marker of neuronal activation) in the midbrain during social defeat stress in conscious Wistar rats. The Wistar rat (an intruder) was moved into a home cage of a Long Evans rat (a resident). After the social-defeated relationship was established between the intruder and the resident, the rats were separated with a wire-mesh in the same cage for 60min. In the intruder rat, blood pressure (BP) and heart rate (HR) transiently increased at the period of first 25-30min and then gradually decreased, whereas both BP and HR maintained at higher level than the pre-stress condition during the defeat stress period. Numerous c-Fos expressions were observed at dorsal region (dPAG) and also at ventral region (vPAG) of the midbrain periaqueductal grey (PAG). It is known that the dPAG is associated to "fight or flight" responses, and that the vPAG is associated with immobility response. The immunohistochemical data are consistent with a recently published result. Therefore, it is likely that the mechanism underlying the response evoked by the social defeat stress involves at least two different PAG regions in the midbrain.

(COI: No)

P3-080

Brain-derived neurotrophic factor immunoreactive vagal sensory neurons innervating the gastrointestinal tract of the rat

Hayakawa, Tetsu; Kuwahara-Otani, Sachi; Maeda, Seishi; Tanaka, Koichi; Seki, Makoto (*Dept. Anatomy, Hyogo College of Medicine, Hyogo, Japan*)

We have determined whether brain-derived neurotrophic factor immunoreactive (BDNF-ir) neurons in the vagal ganglia innervate the gastrointestinal tract. Many BDNF-ir neurons were medium in size and located throughout the jugular and nodose ganglia. When Fluorogold was injected into the wall of the cervical esophagus, many retrogradely Fluorogold-labeled neurons were found in both the jugular ganglion and the nodose ganglion. When Fluorogold was injected into the body of the stomach or applied to the cut end of the subdiaphragmatic vagus nerve, numerous Fluorogold-labeled neurons were found mostly in the nodose ganglion. Double-labeling combining immunohistochemistry for BDNF and retrograde tracing with Fluorogold showed that more than 90% of the neurons in the jugular ganglion and the nodose ganglion projecting to the cervical esophagus expressed BDNF-like immunoreactivity. In the cases of both Fluorogold injection into the stomach and Fluorogold application to the subdiaphragmatic vagus nerve, almost all Fluorogold-labeled neurons in the nodose ganglion expressed BDNF-like immunoreactivity. These results indicated that almost all vagal sensory neurons located in either the jugular ganglion or the nodose ganglion that innervate the gastrointestinal tract are BDNF-ir neurons. Thus, BDNF is thought to be a neurochemical marker for vagal afferent neurons projecting to the gastrointestinal tract.

(COI: No)

P3-081

Intrathecal administration of capsaicin enhances the colorectal motility in rats

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It is known that capsaicin-sensitive sensory neurons modulate gastrointestinal motility. However, their functions in the lumbosacral defecation center are unclear. In the present study, we investigated whether capsaicin acts on the lumbosacral defecation center and affects the colorectal motility. For assessing colorectal motility, rats were anesthetized with alpha-chloralose and the distal colon and anus were cannulated to measure the intracolorectal pressure and propelled intraluminal liquid volume. Stable spontaneous contractions of the colorectum appeared within 1 hour after cannulation in most cases. Intrathecal administration (L6-S1 region of the spinal cord) of capsaicin caused large contractions. The enhancement of phasic contractions after intrathecal administration of capsaicin was accompanied by increased fluid output through the anal cannula. The stimulation evoked by intrathecal capsaicin was prevented if the pelvic nerve was severed. These results suggest that intrathecal administration of capsaicin stimulates colorectal motility maybe by acting on capsaicin-sensitive neurons in the lumbo-sacral defecation center.

(COI: No)

P3-082

Effects of thermal cutaneous stimulation on renal sympathetic nerve activity in rats

Kemuriyama, Takehito; Tandai-Hiruma, Megumi; Ohta, Hiroyuki; Tashiro, Akimasa; Hagisawa, Kohsuke; Nishida, Yasuhiro (*Dept. Physiol., Nat. Def. Med. Coll., Tokorozawa, Japan*)

We have investigated the effects of thermal cutaneous stimulation on renal sympathetic nerve activity (RSNA) in anesthetized rats, to clear the relationship between somatosensory system and autonomic nervous system. Using intraperitoneal urethane (1.2 g/kg), and the left renal nerve was exposed through a left flank incision and dissected free from surrounding tissue under a dissecting microscope in Sprague-Dawley rats. Bipolar silver electrodes were put under the nerve to record. The nerve and electrodes were covered and stabilized with silicone rubber gel. Thermal cutaneous stimulation with a hot pad (approximately 42 degree Celsius) was applied to the forepaws and hindpaws of rats, and RSNA was recorded with a power lab system. After a brief stabilization period, RSNA was recorded for 15 min under thermal cutaneous stimulation. RSNA during thermal cutaneous stimulation to the limbs decreased from the control level. However, efects of RSNA during thermal cutaneous stimulation fluctuated by different thermal temperature of the hot pad. These results suggest that thermal cutaneous stimulation to the forepaws and hindpaws may affect RSNA to indicate autonomic nervous system, but they have various effects by different temperature of thermal cutaneous stimulation. We should try to detect the relationship between the condition and the effects of thermal cutaneous stimulation to the limbs.

(COI: No)

P3-083

Regulation of energy metabolism by intracellular Ca^{2+} signals

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Obesity is a leading cause of life-threatening diseases such as myocardial infarction; therefore, clarifying its molecular mechanisms is therapeutically important. Recent evidence suggests that intracellular Ca^{2+} signals play a critical role in the regulation of energy metabolism. We have previously reported that mice lacking the Ca^{2+} sensor protein NCS-1 (KO), which is important for excitable cell functions, exhibit significant obesity as they age *(Circ. Res. 2011)*. In the present study, we investigated the molecular mechanisms of this phenomenon. Using metabolic cages, we found that food intake and locomotor activity were similar between WT and KO groups. However, energy metabolism and thermogenesis indicators such as O_2 consumption/ CO_2 emission and rectal temperature were significantly lower in KO than WT mice. Indicators of mitochondrial function and number (respiratory rate and the levels of UCP1, PGC-1 α, and VDAC) were also lower. Lipid droplets in both brown and white adipose tissues (BAT and WAT) were dramatically enlarged in the KO group and interestingly, NCS-1 was expressed in the BAT. Metabolomic analyses demonstrated that in the KO group, the metabolites involved in energy consumption decreased in the BAT, whereas those involved in energy storage increased in the WAT, leading to massive obesity. Taken together, these results suggest that NCS-1 is a novel regulator of energy metabolism in adipocytes, and hence can be an important target for the treatment of metabolic syndrome.

(COI: No)

P3-084

GLP-1 suppresses reflex swallowing via the medial part of nucleus tractus solitarius

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Our previous study demonstrated that microinjection of glucagon-like peptide-1 (GLP-1) into the medial part of dorsal vagal complex (m-DVC) suppressed reflex swallowing. However, it has not been clarified the effective site where GLP-1 acts to suppress reflex swallowing among the m-DVC. In the present study, we examined the effective site of GLP-1 to suppress reflex swallowing among the m-DVC by the selective lesion of the dorsal medulla. Swallowing was induced by the electrical stimulation of the central cut end of the superior laryngeal nerve and was identified by the electromyogram lead penetrated the mylohyoide muscle through bipolar electrodes. Each animal underwent one of three lesions: 1) ablation of the area postrema (AP) by suction; 2) electrical lesion of the commissural nucleus tractus solitarius (NTS); 3) electrical lesion of the medial NTS. GLP-1 was injected into the m-DVC. The electric lesion of the medial NTS abolished the suppression of reflex swallowing induced by injection of GLP-1 into the m-DVC. In contrast, ablation of the AP and electrical lesion of the commissural NTS did not abolish the suppression of reflex swallowing induced by injection of GLP-1 into the m-DVC. These results suggest that GLP-1 suppresses reflex swallowing via GLP-1 receptor situated in the medial NTS. This work was supported by JSPS KAKENHI Grant Number 24500630.

(COI: No)

P3-085

Local application of sympathetic nerve blockers around dorsal root ganglion reduces painful behavior in a lumbar radiculopathy model

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The purpose of the present study is to examine the effects of sympathetic nerve blockers in a lumbar radiculopathy model using behavioral study. We prepared a lumbar radiculopathy model, and placed a catheter on the dorsal root ganglion to administer the sympathetic nerve blockers. We administered phentolamine (non selective α-antagonist), prazosin (α_1-antagonist), silodosin(α_1-antagonist), and yohimbine (α_2-antagonist) for 3 consecutive days after 0th, 4th and 11th post-operative days. The concentration of sympathetic nerve blockers was 10 or 100mM. Control rats received vehicle injections. Behavioral analysis using mechanical and thermal stimulation was performed before the operation until 28th post-operative day. Phentolamine and yohimbine reduced painful behavior for 28days. Pain analgesic effect of yohimbine was stronger than that of phentolamine. Prazosin relieved painful behavior almost all experimental periods, however, the effect was weaker than that of phentolamine and yohimbine. In contrast, silodosin had no pain analgesic effect. Phentolamine administerd at 4th and 11th experimental periods, attenuated pinful behavior once generated. The present study showed that sympathetic nerve blockers attenuated the painful behavior via α_2-adrenoceptor. Sympathetic nerve blockers were effective after generation of painful behavior. So we consider that sympathetic nerve blockade of α_2-adrenoceptors may contribute to pain relief in neuropathic pain.

(COI: No)

P3-086

Prediction error responses in the mouse posterior parietal cortex are dependent on protocadherin-αdiversity

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Higher brain areas are responsible for detecting and reducing prediction errors. Previously, we have reported that prediction errors between whisker and visual inputs were detected in the posterior parietal cortex (PPC) of mice. We recorded neuronal activities in PPC using flavoprotein fluorescence imaging. Visual stimulation or whisker stimulation alone hardly activated PPC. However, anti-phase combination of moving grating patterns and whisker stimulation, which is very unlikely in natural environment for mice, produced prediction error responses in PPC. As expected, in-phase combination failed to produce any clear activity in PPC. We have reported that cortical depression and map shifts were induced by prediction errors between visual and whisker inputs in the primary visual cortex (V1) of young mice that had worn a monocular prism goggle, suggesting that the prediction errors detected in PPC were reduced in V1. Clustered protocadherins (cPcdhs) are neuron-specific cell adhesion molecules with multiple clusters. The prism-induced depression in V1 and the prediction error responses in PPC induced by the anti-phase combination of visual and whisker stimulation were impaired in mice with reduced cPcdh-α diversity. These results strongly suggest that the diversity of cPcdh-α is important for the PPC function to detect prediction errors between visual and whisker inputs.

(COI: No)

P3-087

Higher visual cortices responsible for shape recognition in mice

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Higher visual cortices responsible for shape recognition were not identified in mice. We hypothesized that the cortical area involved in shape recognition might be activated by sound stimuli after mice had acquired sound-shape association memory. We have reported that wild-type mice can learn the sound-shape association memory using an M-shaped maze equipped a screen and a speaker. In the present study, we investigated cortical responses to the associated sound stimuli using flavoprotein fluorescence imaging. In trained mice, the responses to the sound stimuli appeared in the auditory cortex and the higher visual area located dorsally to the auditory cortex. Two-photon calcium imaging revealed the presence of shape-specific neurons in this area, indicating that the activated higher visual area plays an important role in shape recognition. Many genetically-manipulated strains of mice are available. Clustered protocadherins (cPcdhs) are neuron-specific cell adhesion molecules with multiple clusters. Wild type mice have 12 clusters (α 1- α 12) of cPcdh- α, while, cPcdh- α 1, 12 mice have only α 1 and α 12 clusters. We found that cPcdh- α1, 12 mice had impaired sound-shape association memory using the M-shaped maze test. Furthermore, the higher visual cortical area was not activated by the associated sound stimuli in these mice.

(COI: No)

P3-088

Divisive normalization during multisensory integration by neurons in macaque area MSTd

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Neurophysiological studies of multisensory integration in single neurons have revealed a set of empirical principles that describe a variety of nonlinear interaction between stimuli from multiple sensory modalities. We previously showed that many of these principles could be explained by a single model based on the divisive normalization mechanism operating in brain regions where multisensory integration is taking place. This normalization model of multisensory integration makes a critical prediction which distinguishes the model from other existing models: that a non-preferred sensory input, which is excitatory on its own, can suppress the response to a preferred input of another modality. We tested this prediction by recording from multisensory neurons in macaque area MSTd that play a critical role for perceiving self-motion by integrating visual (optic flow) and vestibular cues. We show that many MSTd neurons show the diagnostic form of cross-modal suppression. The finding provides strong support for divisive normalization acting at the level of multisensory representations in the brain.

(COI: No)

P3-089

Neuronal activity in the monkey orbitofrontal cortex related to reward value processing during decision-making

Setogawa, Tsuyoshi[1]; Mizuhiki, Takashi[1,2]; Akizawa, Fumika[2]; Kuboki, Ryosuke[2]; Matsumoto, Narihisa[3]; Shidara, Munetaka[1,2] ([1]*Faculty of Medicine, University of Tsukuba, Tsukuba, Japan;* [2]*Grad. Sch. of Comprehensive Human Sci., Univ. of Tsukuba, Tsukuba, Ibaraki, Japan;* [3]*Human Tech. Res. Inst., AIST, Tsukuba, Japan*)

When we make a choice from the alternatives, we consider their values and workloads. To understand the neuronal mechanism of such a decision-making process, we developed a decision-making schedule task and recorded single unit activity from monkey orbitofrontal cortex (OFC) which has been reported to be one of the important brain areas for the reward-guided behavior. Two monkeys were trained to perform a reward schedule task which consists of 1, 2 or 4 trials of visual discrimination to earn 1, 2 or 4 drops of liquid reward. After learning this task, the decision-making schedule task in which two kinds of choice target (CT) were sequentially presented was introduced. The CT brightness and length indicated reward amount and required number of trials, respectively. Then, these two CTs were simultaneously reappeared (choice phase). The monkey was required to choose one of them, and then the chosen reward schedule started. We recorded from 246 neurons in the OFC. In the second CT period, 43.1% (106/246) of the recorded neurons showed correlation between the difference in value of the two CTs and the neuronal firing. Some neurons coded only reward amount (24/246) or workload (5/246) information in the first and second CT period. These results suggest that OFC neurons play an important role in the decision-making by reward value information processing.

(COI: No)

P3-090

Synchronization between respiratory cycles and olfactory neural activations -EEG and fMRI study-

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The link between respiration and olfaction is evidenced explicitly by observed synchronization between respiratory cycles and neural activation of the olfactory circuit during odor perception. Taking advantage of the time-locked nature of inspiration and olfactory processing, electroencephalogram dipole modeling (EEG/DT) has previously been used to identify a cascade of inspiration-triggered neural activity moving from primary limbic olfactory regions to frontal cortical areas during odor perception. In this study, we leverage the spatial resolution of functional magnetic resonance imaging (fMRI) alongside the temporal resolution of EEG to replicate and extend these findings. Brain activation identified by both modalities converged within association regions of the orbitofrontal cortex that were activated from approximately 150ms to 300ms after inspiration onset. EEG/DT was additionally sensitive to more transient activity in primary olfactory regions, including the parahippocampal gyrus and amygdala, occurring approximately 50ms post-inspiration. These results provide a partial validation of the spatial profile of the olfactory cascade identified by EEG source modeling, and inform novel future directions in the investigation of human olfaction.

(COI: No)

P3-091

Effects in EEG and the autonomic nervous system when listening to ringing sound of a wind-bell

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In order to investigate physiological effects of listening to ringing of a wind-bell, we measured EEG and heart rate. Subjects were young healthy 2 men and woman. Two kinds of wind-bell were used, i.e. Nanbu-furin made of iron and glass-furin. As a control sound, monotonic sound generator, digital metronome was used. Average amplitudes of θ, α and β band were not changed during listening to ringing sound. However difference of change width was suppressed by each sound, effectively in occipital regions. Ringing of Nanbu-furin induced suppression of the amplitude difference in 3 bands of 3 persons. Suppression was induced by glass-furin in 2 persons less effectively. Metronome weakly induced the same effect on 2 subjects. These results showed that Nanbu-furin most effectively reduced fluctuation of neural activity in the central nervous system. Reduction of θ and α might show awakening activities and reduction of β might be reduced-neural activities. The parasympathetic nervous activity, analyed by fluctuation of heart rat, was activated and the sympathetic nervous activity was reduced at the beginning of 3 sounds. By using psychological test, the General Arousal Check List (GACL), value of general deactivation was effectively augmented by Nanbu-furin compared to other sounds. These data suggested that listening to Nanbu-furin reduced general neural activity of subjects, namely leading to relaxation in mind, and stimulated the parasympathetic nervous system. This is consistent with the deactivation of arousal level measured by subjective psychological feelings.

(COI: No)

P3-092

Attentional neural network activated by a simple task design and the effect of genetic variations in human brain

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Attention is the climax of mental integration and closely related to consciousness. In order to explore attention in humans, we should use a simple behavioral task. We employed ANT (attention-networks test) that has also been applied to fMRI brain imaging (Fan J. et al., 2005). The task was to request participants to report the direction of an arrow projected on a screen. By using ANT we are able to detect activations in brain associated with alerting by presenting a cue, orienting by spatial cues, and conflict resolving by simultaneously presenting disturbing franker arrows heading towards the other end. Several significantly activated areas have been detected in alerting and orienting. These areas are almost in accord with the dorsal frontoparietal network proposed by Corbetta et al. (2008), involving dorsal parietal and dorsal frontal cortices. Visual association areas were also activated in alerting possibly by the top-down signal. No significant activation was detected in conflict resolving at present (14 subjects). Brain activities are "intermediate" phenotypes situated between genes and human behavior. To assess the intermediate phenotypes, brain imaging by using fMRI is most suited. We are investigating several attention-related and unrelated genetic variations (SNPs) to find the effect of genes on the activation pattern detected.

(COI: No)

P3-093

Keratan sulfate in cortico-basal ganglia circuits is involved in acquired vocalization

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The brain system corresponded to skill movements are constructed during the developments. The learned vocalizations including human speech are acquired in postnatal age. They have sensitive or critical period in juvenile stage. Birdsong learning in the zebra finch occurs during a sensitive period, and the involved brain areas are identified as "song system" in cortex and basal ganglia. However neurochemical properties which clearly discriminate song system from the other circuits remained unclear. Here we report that the highly sulfated keratan sulfate is specifically expressed in song system including HVC (the proper name), robust nucleus of the arcopallium (RA), lateral magnocellular nucleus of the anterior nidopallium (LMAN), and Area X. They are distributed in the extracellular matrix especially in supra cell membrane area. Synaptic connections are formed through their defected area. We evaluated the differences of the keratan sulfate intensities among developmental stages and sex. We also examined the molecular properties by the use of Western blotting and chondroitinase. Our results imply that the highly sulfated keratan sulfate are involved in maturation of the learned vocalization. These findings offer new information to facilitate an understanding of the formation of the experience-dependent vocal learning including human language.

(COI: No)

P3-094

Cell-type-specific sustained activity of LIP neurons during covert search but not in overt saccade in a visual search paradigm

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When a target object is embedded in a complex visual scene and less salient, we should carefully search for that target. Model studies suggest that a degree of carefulness in discrimination tasks might be neuronally set by adjusting the criterion level for decision making. However, this assumption is not consistent with the results of neurophysiological studies. To address this issue, we recorded single-neuron activity in the lateral intraparietal area (LIP) when monkeys performed a color-singleton search task involving both target and no-target (catch) trials. Monkeys had to make a saccade to the target in the target trials, whereas they had to maintain fixation throughout the trial in the catch trials in which no target element was presented in a search array. We found that a part of neurons exhibited the stronger activity when the target appeared in the receptive field in the target trials (acceleration-type neurons), whereas another part of neurons exhibit the stronger activity especially when the monkeys successfully maintain the fixation in the catch trials (break-type neurons). The enhanced activity of break-type neurons disappeared when the monkeys simply fixated into the spot stimulus (not fixation cells) and when they erroneously made a fixation break by making saccades in the catch trials. These findings suggest that break-type neurons activated especially when the monkeys make careful covert search during visual search.

(COI: No)

P3-095

The anxiety- and fear-related behavior on the maternal separated mice

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Early life stress is known to induce long-term alterations in emotional and anxiety-related behaviors. Rodent models of neonatal maternal separation (MS) stress have been used to explore the effects of early stress on changes in affective and cognitive behaviors. MS are associated with structural changes in brain regions linked to cognition and mood regulation. Here, we studied the effects of MS on the alteration of neurogenesis in linbic system and anxiety-related behavior on C57Bl/6 mice. The MS was performed daily for 3 hr from P1 to P14 and behavioral test was started at 10 weeks of age. We used a battery of stress and anxiety-related behavioral tests in C57Bl/6 mice. 1. The open field test, which measures the basal anxiety level, showed that MS mice tended to spend shorter in the center area, although total moving distance did not differ. 2. The acoustic startle response induced by the sudden loud tone stimulus was significantly elevated in MS mice. 3. The contextual and cued fear conditioning test provides a measure of memory by assessing a memory for the association between an aversive stimulus and a tone stimulus. Ms mice showed decreased fear conditioning to the context and the tone compared to control. Especially, freezing time during tone stimulus was significant attenuated in MS. Thus in MS mice, fear memory was impaired, although startle response was elevated. 4. Neurogenesis in the limbic system was increase in MS mice. These results suggest that neonatal MS treatment enhances the neurogenesis and alters the anxiety- and fear-related behavior.

(COI: No)

P3-096

The 5-HT3 receptor is essential for exercise-induced hippocampal neurogenesis and antidepressant effects

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Exercise has a variety of beneficial effects on brain structure and function, such as hippocampal neurogenesis, mood and memory. Previous studies have shown that exercise enhances hippocampal neurogenesis, induces antidepressant effects, and improves learning behavior. Brain serotonin (5-hydroxytryptamine, 5-HT) levels increase following exercise, and the 5-HT system has been suggested to play an important role in these exercise-induced neuronal effects. However, the precise mechanism remains unclear. In this study, analysis of the 5-HT type 3A receptor subunit-deficient (htr3a-/-) mice revealed that lack of the 5-HT type 3 (5-HT3) receptor resulted in loss of exercise-induced hippocampal neurogenesis and antidepressant effects, but not of learning enhancement. Furthermore, stimulation of the 5-HT3 receptor promoted neurogenesis. These findings demonstrate that the 5-HT3 receptor is the critical target of 5-HT action in the brain following exercise, and is indispensable for hippocampal neurogenesis and antidepressant effects induced by exercise. This is the first report of a pivotal 5-HT receptor subtype that plays a fundamental role in exercise-induced morphological changes and psychological effects.

(COI: No)

P3-097

Possible mechanisms of glucose-induced facilitation of spatial memory and hippocampal plasticity

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We have previously reported that intrahippocampal injection of 7 mM glucose, which is similar to the glucose concentration of the cerebrospinal fluid during food intake, facilitates spatial learning and memory in rats. The high glucose pre- and postsynaptically enhances basal synaptic response and tetanus-induced long term potentiation in the rat Schaffer collateral/commissural pathway through increases in CAMKII and PKC autophosphorylations. In the present study, we further sought to clarify the cellular mechanisms of the glucose effects using neuronal (N2A) cell lines. When glucose was increased from 3.5 mM to 7 mM, N2A cells showed an increase in expression of brain-derived neurotrophic factor (BDNF) along with the enhanced phosphorylation of AKT (PKB) and CREB. Interestingly, the glucose-induced upregulation of *BDNF* was blocked by the knock down of CREB using lentiviruses encoding short hairpin-RNA against CREB, while high glucose increased CREB recruitment onto the *BDNF* promoter. Furthermore, glucose stimulation reduced histone deacetylase (HDAC) recruitment near the *BDNF* promoters and an HDAC inhibitor, suberanilohydroxamic acid (SAHA) increased *BDNF* expression. These findings, taken together, suggest that glucose enhances spatial learning and memory at least partly through an epigenetic regulation of BDNF gene expression.

(COI: No)

P3-098

Hippocampal plasmalogens regulate memory related gene expressions via modulating the BDNF-TrkB signaling

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Plasmalogens (Pls) are unique glycerophospholipids carrying the vinyl ether linkage at the sn-1 position of the glycerol backbone. Although it has been reported that Pls content is reduced in the brain of Alzheimer s disease (AD) patients, the precise function of Pls is mostly elusive. To understand the impact of the Pls reduction in the brain, we constructed lentiviruses delivering shRNAs against the Pls synthesizing enzyme, glyceronephosphate O-acyltransferase. Intra-hippocampal injection of the shRNAs in mice reduced the Pls content in the hippocampus, impaired Morris water maze task and reduced hippocampal expression of memory related genes such as BDNF, Trk B, Synapsin-1, Synapsin-2, Synaptotagmin-1, PSD-95, CamKII-α and Homer-1. Since BDNF and its receptor TrkB could regulate expression of those genes, we investigated the role of Pls in the BDNF-TrKB signaling. The function of TrkB receptor was known to be dependent on its localization in the lipid rafts, where we confirmed that Pls were also highly present. Interestingly, when Pls were reduced in the hippocampus the TrkB receptor localization was shifted from raft to non-raft fraction. Furthermore we have observed that Pls diet in mice for 6 weeks increased the expression of BDNF and enhanced the memory task, which was blocked by the knock down of BDNF/TrkB. We therefore, propose that hippocampal Pls are necessary for normal memory task through maintaining the BDNF-TrkB signaling in the hippocampus.

(COI: No)

P3-099

Somatomotor integration underlies tactile learning

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Animals actively acquire sensory information via their interactions with the environment. This is especially evident in active touch, where self-generated movements drive tactile inputs. However, due to the lack of an efficient learning task, the mechanisms of tactile learning have yet to be elucidated. We created a tactile-version of an object recognition task, named the object-floor recognition task (OFRT). This task utilizes floor texture as tactile cue to allow mice to discriminate between the same objects placed on different floors. It was observed that after experiencing only one floor on Day 1, on Day 2, the mice preferred the object on the novel floor compared to object on the familiar floor. This learning did not require room light, indicating that the mice could discriminate floors using tactile cues alone. Optogenetic silencing of the primary somatosensory (S1) hindlimb area impaired memory acquisition. Similarly, silencing of the projections from the secondary motor cortex (M2) to S1 or the S1-M2 pathway also impaired memory acquisition. These results suggest that direct mutual somatomotor projections are important for the perceptual learning for discrimination of tactile texture.

(COI: No)

P3-100

Molecular mechanism of the sensitive period of filial imprinting

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The timing and duration of the sensitive period for learning has been believed to be developmentally fixed and unable to be changed. However, there is now reason to believe that the sensitive period can be flexible in terms of the timing and duration. Filial imprinting in birds is the process of forming a social attachment during a sensitive or critical period, restricted to the first few days after hatching. Imprinting is considered to be part of early learning to aid the survival of juveniles by securing maternal care. We showed that the thyroid hormone determines the start of the sensitive period. Imprinting training in chicks (Gallus gallus domesticus) causes rapid inflow of thyroid hormone. The hormone thus initiates and extends the sensitive period to last more than 1 week via non-genomic mechanisms. It can also confer what we term "memory priming (MP)" to prime subsequent learning. Once chicks have achieved MP, it is maintained for long periods. Even in non-imprinted chicks whose sensitive period has ended, exogenous thyroid hormone enables imprinting. It is possible that the sensitive period closes only if MP is not conferred at an appropriate time of development. Under natural conditions, chicks will learn spontaneously with the help of parents and siblings. In a sense, the closing of the sensitive period for learning may not exist under usual physiological conditions probably because the sensitive period does not close as long as MP is acquired. Our study elucidates the critical role of imprinting to subsequent learning as being governed by the acute action of thyroid hormone.

(COI: No)

P3-101

Neuronal activity in the monkey prefrontal cortex during a temporal classification task

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To address the neuronal mechanism for interval timing in the prefrontal cortex (PFC), we examined neuronal activity in the PFC of a monkey during a temporal classification task. In the task, a visual cue was presented on the center of the monitor from 0.8 to 4.8 sec. Following a 1 sec delay period, the subject was required to press the proper key according to the classification of cue duration; the right, center, and left keys for long (3.2-4.8 sec), middle (1.6-2.4 sec), and short (0.8-1.2 sec) categories, respectively. For the spatial control of key selection and movement, the subject also performed a spatially cued delayed response task, in which a visual stimulus spatially cued the proper key. Of 277 PFC neurons we recorded, 124 neurons were related to the temporal classification task. Two types of task-related activities were interesting. The first one was phasic activity during the cue period with constant peak time after the cue onset. The peak times of these activities broadly distributed with a few peaks including 1.2 and 1.8 sec after the cue onset. These phasic cue activities might function to filter current cue duration with the peak time. The second one was phasic activity during the delay period, which changed according to the cue duration categories. The delay activity might represent temporal classification results of cue duration. These results suggest that the PFC contributes to a classification process of visual signal duration.

(COI: No)

P3-102

Effect of mother-infant interaction on the relationships between amygdalar dopamine release and open-field behaviors

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Early-weaned rodents exhibited changes in behavioral and emotional traits (Shimozuru et al, 2007) and myelin formation in the anterior basolateral amygdala (aBLA; Ono et al, 2008), which the prefrontal efferents terminated reportedly. We previously found that early-weaned SD male rats (postnatal day [PND] 16) exhibited lower paired-pulse facilitation than did late-weaned rats (PND 30), in the prefrontal-aBLA pathway of urethane-anesthetized juvenile rats. Rosenkranz & Grace (e.g., 2001) suggested the efferents regulated the BLA inhibitory interneurons to suppress the affective response of the BLA driven by the sensory cortex: Dopamine (DA) attenuated this regulation and synergistically enhanced the sensory cortex-driving response. We found here, by using in vivo microdialysis, the amygdalar DA were statistically 2.5 times higher for the basal release concentration at home cage in the early-weaned group: The relative reactivity tended to be higher at transfer timing from the home cage to open-field and vice versa. On the contrary, locomotor and rearing tended to be lower at either the timing in the early-weaned group. We will show correlations between the DA release and associated open-field behaviors on the mother-infant interaction at the meeting. The study was supported by KAKENHI (M.T).

(COI: No)

P3-103

Optogenetic silencing of serotonin neurons on escalated aggression in male mice

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Aggression is ethologically important behavior for many animals. However, if the level of aggression becomes escalated, that behavior is no more adaptive and it is important to understand neural mechanism of escalated aggression which is relevant to preclinical and clinical concerns. To escalate aggressive behavior of male mice, we used social instigation procedure in which test animal can see the existence of rival in the protected cage but cannot physically attack it. In vivo microdialysis showed that serotonin (5-HT) release was increased by social instigation and during escalated aggressive behavior in the dorsal raphe nucleus (DRN) and the medial prefrontal cortex. On the other hand, there was no change of 5-HT release during basal species-typical aggression. Therefore, an activation of 5-HT system may be involved in escalated aggression induced by social instigation, but not in species-typical aggression. To examine this possibility, we manipulate activity of 5-HT neurons by optogenetics during aggressive behavior of male mice. KENGE-tet transgenic mice that express inhibitory archaerhodopsin (ArchT) specifically on the 5-HT neurons were used. As expected, there was no effect of optogenetic silencing of 5-HT neurons during species-typical aggression. The effect of optogenetic silencing on escalated aggression will also be presented.

(COI: No)

P3-104

Resting-state brain networks related to personality traits

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A number of brain regions have been suggested to be related to personality traits; however, their neural correlates remain largely unknown. In this study, we determined the relationship of personality estimates with a network centrality of the brain as measured by resting-state fMRI. Personality estimates were obtained from Cloninger's Temperament and Character Inventory (TCI). Big-Five scores were estimated by TCI scores. We used a 3-Tesla MRI (PHILIPS) to obtain T2*-weighted functional (5 min x 3) and T1-weighted structural images from healthy right-handed male subjects (N=89, 18-24 years old). Subjects were asked to stay awake with their eyes closed. BOLD signal was preprocessed through SPM8 and in-house software developed on MATLAB (Mathworks). An adjacency matrix was obtained from all gray matter voxels (down sized to 6x6x6 mm) to determine various network centrality measures (such as degree centrality) for each voxel. We examined whether seven TCI scores and Big-Five estimates were significantly related to these centrality measures ($p<0.05$, FWE corrected by factorial design performed on SPM8). We observed in detail the score-specific spatial distribution of significant voxels not only in the cerebral cortex, but also in subcortical structures. Our results suggest that the personality traits are represented in the resting-state brain networks that involve specific cortical and subcortical regions related to each trait.

(COI: No)

P3-105

Postweaning period is critical for the effect of MSG on social behavior in ADHD model rat

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Attention-deficit/hyperactivity disorder (ADHD) is characterized by hyperactivity, impulsivity and inattention. Dysfunction of mesocorticolimbic dopaminergic (DAergic) system such in the amygdala and prefrontal cortex is related to emotional regulation in ADHD. We reported that oral intake of monosodium L-glutamate (MSG), a taste substance for umami, for 5 weeks from postnatal day 25 (P25) to P60 altered social behavior in ADHD model rat (SHR), and dopamine receptor expression (D1R and D2R) was significantly increased in MSG-treated amygdala. In this study we investigated the critical period in MSG effect on social behavior. SHR rats (P25) were housed in an isolated condition (one rat per cage) and treated with 0.6% MSG for various periods until P60: early-treated group (P25-P40), late-treated group (P40-P60), all-period group (P25-P60) and non-treated group. Early-treated group decreased the number of riding (parameter of aggression to unfamiliar rat) compare with control group, which is the same level as all-period group. However, no significant difference was found between late-treated group and non-treated group. Early-treated group also showed faster reduction in the sniffing time (parameter of exploration), indicating that the rats easily accept to unfamiliar rat. Data suggest that MSG intake during early life (P25-P40) may affect mesocorticolimbic DAergic system, relating to social behavioral changes that persist into adulthood.

(COI: No)

P3-106

Effects of aging and idiopathic Parkinson's disease on tactile temporal order judgment

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It is generally accepted that the basal ganglia play an important role in interval timing that requires the measurement of temporal durations. By contrast, it remains controversial whether the basal ganglia play an essential role in temporal order judgment (TOJ) of successive stimuli, a behavior that does not necessarily require the measurement of durations. To address this issue, we compared the effects of idiopathic Parkinson's disease (PD) on the TOJ of two successive taps delivered to each hand, with the arms uncrossed in one condition and crossed in another. In addition to age-matched non-PD participants, we examined young healthy participants. There was no significant difference between PD and non-PD participants in any parameter of TOJ under either arm posture, although reaction time was significantly longer in PD compared with non-PD participants. By contrast, the effect of aging was apparent in both conditions. With their arms uncrossed, the temporal resolution in elderly participants was significantly worse compared with young participants. With their arms crossed, elderly participants made more errors at longer intervals (~1 s) than young participants, although both age groups showed similar judgment reversal at moderately short intervals (~200 ms). These results indicate that the basal ganglia do not play essential roles in tactile TOJ and that the effect of aging on TOJ is mostly independent of the dopaminergic systems.

(COI: No)

P3-107

Cortical mechanisms of top-down control for precise sensory perception

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Little is known about mechanisms how higher cortical areas control sensory processing. Here, we report the identification and characterization of a neural circuit mediating top-down control in the mouse somatosensory system. The circuit comprises a long-range recurrent horizontal projection between primary somatosensory cortex (S1) and secondary motor cortex (M2). Physiological recordings revealed that M2 top-down input provides temporally coincident input to the upper and lower layers of S1, evoking dendritic spikes and large firing of layer 5 neurons. Contrarily, optogenetic inhibition of top-down input in S1 decreased L5 firing and lead to inaccurate perception. These data demonstrate that bottom-up and top-down inputs to S1 are necessary for accurate sensory perception.

(COI: Properly Declared)

P3-108

A new stop-signal task to explore inhibitory function in operant learning to habituation process

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To control our behaviors properly, we need not only a function to execute a behavior but also a function to inhibit it (inhibitory function). It is becoming clear that the execution of behavior is controlled by a network of the cerebral cortex and basal ganglia, which shows dynamic shift in an operant learning to habituation process. On the other hand, little is known about how inhibitory function is controlled in the course of operant learning to habituation process. To elucidate the mechanism of inhibitory function at cellular and network level, it is necessary to record neuronal activity from animals (e.g., rats) working at a behavioral task which requires inhibitory function. Stop-signal task is often used to assess inhibitory function; however, (1) this standard task takes them a very long period for learning/habituation, and (2) it might be hard to record neuronal activity stably and precisely in usual freely-moving animals during their task performance. To address the issues, we constructed a novel stop-signal task for rats in a head-fixed condition. We took advantage of our spout-lever system (Kimura et al. 2012) to shorten the period of learning/habituation effectively. Thereby, we established the stop-signal task that the head-fixed rats learned to perform in three weeks. This novel stop-signal task will enable us to elucidate the mechanism of inhibitory function in the operant learning to habituation process at a cellular and network level.

(COI: No)

P3-109

Salicylate-induced neural changes of the FM function in the primary auditory cortex of guinea pigs observed by optical recording

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The influence of salicylate on neural activities to frequency-modulated (FM) sounds with different FM sweep rates in the primary auditory cortex (AI) of the guinea pig was investigated using optical imaging with a voltage-sensitive dye (RH795). Eight guinea pigs were anesthetized with ketamine (80 mg/kg) and xylazine (40 mg/kg). Activity patterns to the FM sounds (upward and downward linear sweep: 0.5-16.5 kHz in 16-160 ms duration or FM sweep rate 0.1-1 kHz/ms) and tones (0.5, 16 kHz) at 75 dB SPL were recorded from the AI on both sides before (control) and 8 hours after the intraperitoneal injection of 200 mg/kg salicylate. The peak of the response to the 0.5-kHz tone showed the maximum amplitude at the 40- or 160-ms duration and the peak amplitude of the 0.5-kHz frequency-band (FB) was larger than other FBs independent of the duration. The peak of the response to the FM sound showed the maximum amplitude at the 16-ms duration in the 16-kHz FB and the peak decreased when the FM duration increased up to 64 ms after salicylate injection, but under the control condition up to 40-ms duration. The difference of the amplitudes between the 16-kHz and another FB at the 16-ms duration was larger for the upward FM sound than the downward FM sound under both the salicylate and control condition.

(COI: No)

P3-110

Anti-inflammatory protein TSG-6 secreted by MSCs reduces neural damage and improves memory defects after traumatic brain injury in mice

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Traumatic brain injury (TBI) causes multiple long-term defects including a loss of working memory that is frequently incapacitating. Administrations of mesenchymal stem/stromal cells (MSCs) previously produced beneficial effects in models of TBI as well as other disease models. In several models, the beneficial effects were explained by the MSCs being activated to express TSG-6, a multifunctional protein that modulates inflammation. Intravenous human MSCs or TSG-6 decreased neutrophil extravasation, expression of matrix metalloproteinase 9 by endothelial cells and neutrophils, and the subsequent blood brain barrier leakage. Administration of TSG-6 also decreased the lesion size at 2 weeks. Importantly, the acute administration of TSG-6 within 24 hour of TBI was followed 6 to 10 weeks later by improvements in memory and the number of newly born-neurons. The data suggested that acute administration of TSG-6 may be an effective therapy for decreasing some of the long-term consequences of TBI.

(COI: No)

P3-111

Pre-weaning behavioral patterns in prenatal bisphenol A treated rats

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Bisphenol A (BPA) is well known as one of the environmental endocrine disruptors. Past our study showed that pre- and postnatal administrations of low-level BPA impaired the gender differences in open-field behaviors in rats. BPA also induced the depression-like behavior and enhanced the response to the predator odor in adult age. In this study, we focused on the behaviors in the pre-weaning period. We administered low-level BPA (1.5 mg/kg/day) to prenatal rats, examined the behaviors in 8 days and in 20 days old. In this, we designed the methods using the predator odor (fox odor). In 8 days old, a twitching, pivoting, head-moving, crawling and an immobility were examined. There were no significant effects in the gender and in the treatment. In the presence of fox odor, the decreasing of head-moving and the increasing of immobility were revealed in all groups. Number of animals which displayed the crawling was decreased by the fox odor only in BPA rats. In 20 days old, a rearing, locomotor activity, grooming and the immobility were examined. In the control rats, gender difference was revealed in the locomotor activity, then, this difference was not displayed in the BPA rats. Under the fox odor, the rearing and the locomotor activity were decreased, the immobility was increased in all groups. Corticosterone levels after exposure of fox odor, were increased in all groups. It suggested that stress-induced behavioral alterations were observed in both 8 days and 20 days old. The odor-related changes by BPA treatment were revealed in the crawling, but, generally not so remarkable in other parameters.

(COI: No)

P3-112

Effect of a rubber hand on cross-modal dynamic captures

Wada, Makoto; Ide, Masakazu (*Dev Disorder Sect, Dept Rehab Brain Func, Res Inst of NRCD, Tokorozawa, Japan*)

Apparent motion is sometimes affected by other sensory modalities ('cross-modal dynamic capture', CDC); and tactile temporal order judgment (TOJ) is known to be affected by visual apparent motion. Furthermore, hand images enhance temporal integration of visuo-tactile inputs during TOJ and temporal recalibration. Here, we investigated effects of the hand presentation on the CDC effects during tactile TOJ. In front of a participant, a rubber hand was placed at a forward direction (Forward condition); and it was placed at an inverted direction in the other session (Inverted condition). Participants (n = 12) were required to judge temporal orders of the tactile stimuli to an index finger and ring finger of their hidden right hands and to ignore visual stimuli from LEDs that were placed on corresponding fingers of the rubber hand. When incongruent visual stimuli were delivered, participant's judgment was notably reversed at moderately short intervals during Forward condition. In contrast, amounts of the reversals were significantly decreased during Inverted condition ($P < 0.01$). Furthermore, the changes (Inverted / Forward) were negatively correlated with each Autism Spectrum Quotient ($R = -0.64$, $P < 0.05$). Our present results suggest that the rubber hand corresponding to one's own hand facilitates visuo-tactile CDC effect; and present results might indicate relationships between the multisensory processing and social cognitions.

(COI: No)

P3-113

Effects of periodicity of brush stroking on the rubber hand illusion

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Participants experienced illusory ownership sensation of a dummy hand when the dummy hand and participants' hidden hand were synchronously stroked by brushes (Rubber Hand Illusion, RHI). Previous studies have revealed that synchronous stroking to participants' and dummy hands enhances the RHI rather than asynchronous stroking, and acceptable range of synchronicity to occur the illusion is relatively large (300 ms). However, to date, effect of periodicity of stroking has not been investigated yet. In this study, we examined whether the periodicity of stimulation influences occurrence of the RHI with PC controlled brushes. Participants (N = 24) experienced two times three conditions: 1. Cyclic stroking, 2. Non-cyclic stroking, 3. Asynchronous stroking (e.g., 6 sessions in total). We asked them subjective feelings as for ownership sensation of a dummy hand and amounts of proprioceptive drifts induced by the RHI after each sessions. We found that both cyclic and non-cyclic stroking vividly caused the RHI. However, when non-cyclic stroking was delivered, the ownership sensation was significantly decreased in second session compared to the conditions with cyclic stroking ($p < 0.05$). Amounts of the proprioceptive drifts were not different between the two conditions. These findings indicate that the periodicity of the stimulation intervals as well as the synchronicity of stimulation can influence the ownership sensation of the dummy hand.

(COI: No)

P3-114

A rubber hand experiment using an EMG controlled robotic arm

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Feeling ownership of our limbs represents a fundamental aspect of self-consciousness, and in some circumstances, the feeling is extended out of our own body, as in the rubber hand illusion (RHI, Botvinick and Cohen, 1998). In our rubber hand experiment, we used an in-house electromyography (EMG) controlled robotic arm, and evaluated sense of agency (SA) and sense of ownership (SO) (Kalckert & Ehrsson, 2014). A plastic board was placed horizontally in front of participants (n=15), and each participant placed their right hand under the board. EMG signals were recorded from the participants' arm to control the robotic arm, which was placed above the board. The robotic arm and a participant's own hand are synchronously or asynchronously stroked by paint-brushes. After the experiment, SA and SO were evaluated by subjective ratings (-3 to +3). SO was also measured by a proprioceptive drift. Subjective ratings (SA/SO) were significantly greater than 0 under the synchronous condition ($p < 0.05$). When the participants' arm was synchronously stroked, significantly bigger proprioceptive drift to the robotic arm side (SO) was observed. These results suggest that rubber hand illusion was induced by using an EMG controlled robotic arm.

(COI: No)

P3-115

Real-time change of neural activity in the hippocampal CA1 and medial prefrontal cortex before, during, and after the exposure to a specific episode

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The episodes of the first love or sexual relationship remain in memory strongly. To monitor the process of episodic memory, we recorded neuronal activity in the hippocampal CA1 and medial prefrontal cortex in freely moving young male rats before, during, and after the first encounter with young female rats for 10 min in the male rat's home cage. A few minutes after the female rat was placed in the male rat's home cage, CA1 neurons fired with high frequency (~100Hz) for seconds several times. In addition, the ripple-like events and the amplitude of miniature EPSC and IPSC were increased after the female rats was taken out. Furthermore, the ripple-like events came to be observed in the medial prefrontal cortex 30 min after the female rat was taken out, and which were synchronized with ripple-like events in the CA1. The episode of the first encounter with female rats changed neural activity and synaptic plasticity in the CA1 of male rats. The synchronization of ripple-like events between the CA1 and medial prefrontal cortex might have a role in memory consolidation. These observations shows a real-time change in the neural activity in the hippocampal CA1 and medial prefrontal cortex before, during, and after a specific episode, and could be involved in the process of episodic memory formation.

(COI: No)

P3-116

New periodical components in EEG frequency spectrum observed by corresponding electrode near T5/6 of the brain stem

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Human electroencephalogram (EEG) reflects their brain activities, and it has been widely used in clinical diagnoses as well as basic studies for neurosciences. However, the under -lying mechanism of EEG still not yet well understood. Especially, it must be elucidated how are composed of the electrical activities of the brain stem, as well as those of the cortical and subcortical brain. To address this issue, we here search for areas of normal body, near the brain stem where any localized activities may be recorded or not. Their resting state EEG was recorded from the electrodes placed not only at Cz and Oz, but also at the scalp bow-edge behind the auricle near T5 (left side) and T6 (right side of brain), which is defined here as T5'/6', and really succeeded to obtain an interesting waveforms. We have also investigated the wave signals using the FFT spectrum analyzer, and found that both of T5' and T6' showed the same spectral results of sharp line peaks in low frequency range (2-8Hz) separated almost equivalent frequency spacing of 1Hz. Such components were little influenced by eye-opening and changing the posture. We also observed the activity of nebula by attaching an electrode at inion, and found that almost similar frequency line-spectra. On the other hand, the spectra observed by Cz and Oz electrodes showed only typical alpha peak without any such line peak series. These frequency spectra may attributed to electrophysiological activities derived from the mid-brain and the brain stem.

(COI: No)

P3-117

Relation between hippocampal sharp wave ripple events and prefrontal theta activities during delayed reinforcement task

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Hippocampal sharp-wave ripples are component of the local field potential and characterized short oscillatory activity at high frequency (about 100-250Hz) during slow wave sleep. Importantly, the sharp-wave ripple events are well correlated with memory formation. These events are observed during the awake quiescent state immediately before or after the learning task as well. On the other hand, the interaction between the hippocampus and the prefrontal cortex is an important for memory consolidation. However, the interaction of the hippocampal ripple and prefrontal activities during learning stage has not been established. In the present study, we recorded the hippocampal local field potential during the delayed reinforcement task, one of memory retention test, and analyzed the sharp-wave ripple events. After the recording, the ripple events were determined during delay period of the task and were analyzed separately for correct trials and incorrect trials. In the correct trials, the number of the ripple events immediately before reward cue during delay period was increased and the peak of the number of events was correlated with performance of the task. In contrast, the number of ripple events during incorrect trials showed no significant change. In addition, prefrontal theta activities with hippocampal ripple events were increased at term of immediately before reward cue. The ripples before reward cue may affect prefrontal activities for a reward related memory recall or memory consolidation.

(COI: No)

P3-118

Characteristics of Postural Control Disturbance in Rats with Cerebellar Vermis Lesion: a Study using Posturography Technique

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We aimed to reveal characteristics of postural control disturbances in rats with cerebellar vermis lesion using a posturography technique. For this purpose, under pentobarbital anesthesia, the cerebellar vermis of male rats with a weight of about 300g (n = 5) was removed using a suction device. We measured changes of center of pressure (COP) of the animals during floor inclination in four directions, i.e. left-right and antero-posterior directions, at an angle from 0 to 30 degrees with different angle velocities from 1.8 to 15 degree/sec. In 2 of 5 animals, we also made chronic recordings of EMG activities of extensor muscles of fore- and hind-limbs together with measurements of COP changes. All measurements were made in 2 days before lesion and 2, 7, 14 days after lesion. In 2 days after lesion, most of the animals showed remarkably increases of COP changes during floor inclinations, especially in antero-posterior directions, thus indicating disturbances of postural control. In addition, they also exhibited uncoordinated EMG activities during postural control. These tendencies became much remarkable in 7 and 14 days after lesion. These results suggest that the cerebellar vermis is closely related to the postural control in antero-posterior direction in rats.

(COI: No)

P3-119

Aerobic treadmill training prevents the obesity caused by gene disruption of Ca^{2+}-sensor protein in mice

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Regular exercise with appropriate nutrition can help reduce body fat as well as protect against chronic failure like cardiovascular diseases. Genetic disruptions sometimes induce obesity via unknown mechanism in mice. The neuronal Ca^{2+}-sensor 1 (NCS-1) is one of such genes. Gene knockout (KO) of NCS-1 results in obesity, probably via reduced basal metabolism. NCS-1 is expressed in excitable cells like neurons and hearts, and regulates many physiological functions. In this study, we examined whether proper exercise can prevent obesity in NCS-1 KO mice. A group of age-matched wild-type (WT) and KO mice were subjected to 8-weeks (5 days/week) aerobic treadmill running (slope 10 degree, 15-20 m/min, 60 min) and others were placed to sedentary. In both WT and KO mice, compared to sedentary, training groups exhibited outstanding exercise effects; high running ability at lactate threshold level and enlargement of tibialis anterior muscle fibers. However, echocardiography showed no apparent sign of athletic heart. In both WT and KO mice, training much decreased the body weight and the epididymal fat weight. Training was very effective to reduce the size of lipid droplets in brown and white adipose tissues within interscapular fat, indicating increased fatty acid consumption by training. These findings suggest that aerobic training is very effective to prevent obesity even in genetically inherited obese constitution.

(COI: No)

P3-120

Meso-limbic outflow toward descending motor pathways in monkeys

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Most people had experiences that led to better motor performance when they had higher motivation. In addition, the result of better performance is thought to boost one's motivation. Recently, we found that the meso-limbic system involved in processing of motivation increased the activity in association with that of the primary motor cortex during recovery course from the spinal cord injury in monkeys. Motivation might be a key issue for motor performance and functional recovery. However, it is unclear how the activity of the meso-limbic system affects that of motor-related areas and motor outputs. To clarify this question, evoked-electrocorticogram and upper limb muscle responses induced by electrical stimulation of the meso-limbic system were obtained from two sedated monkeys. Electrical stimulation was applied to the ventral tegmental area (VTA) or the nucleus accumbens (NAc), which are included in the meso-limbic system. The result showed that VTA stimulation induced stimulus-dependent responses not only in the orbitofrontal cortex (OFC) but also in the primary motor cortex (M1). In contrast, NAc stimulation induced responses not in M1 but in OFC. In addition, repetitive stimulation of VTA induced excitatory responses in upper limb muscles. These results suggest that the meso-limbic system can modulate the M1 activity and motor outputs and demonstrated neural substrate for emotional control of motor outputs.

(COI: No)

P3-121

Contribution of the activity of frontal eye field fixation neurons to the suppression of saccades and smooth pursuit eye movements in the monkey

Izawa, Yoshiko; Suzuki, Hisao (*Dept Systems Neurophysiol, Grad Sch Med, Tokyo Medical and Dental Univ, Tokyo, Japan*)

Focal electrical stimulation in the frontal eye field (FEF) suppresses the generation of saccadic and smooth pursuit eye movements at an intensity lower than the threshold for eliciting electrically evoked saccades. We previously found a localized area of the FEF in the caudal part of the arcuate gyrus facing the inferior arcuate sulcus in which stimulation suppressed the generation of saccades and pursuit in bilateral directions and also where fixation neurons discharging tonically during fixation were concentrated. Fixation neurons usually showed a reduction in activity during saccades. The present study analyzed the activity of fixation neurons in the FEF during pursuit in trained monkeys. Fixation neurons showed a variety of discharge patterns during pursuit, ranging from a decrease in activity to an increase in activity. Of these, more than two thirds of fixation neurons were found to show a reduction in activity during pursuit toward ipsilateral or bilateral directions. When catch-up saccades during the initiation of pursuit were eliminated by step-ramp target routine, the reduction in activity of fixation neurons survived. The present results suggest that fixation neurons in the FEF may contribute to the generation of pursuit suppression. These findings support the idea that this type of fixation neuron assembly as a whole in the FEF may be part of a more generalized visual fixation system through which suppressive control is exerted on pursuit as well as saccades.

(COI: No)

P3-122

Single-unit activity in supplementary motor area of Japanese monkeys walking on a treadmill

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To further understand cortical mechanisms for controlling bipedal (Bp) gait in humans, we recorded single-unit activity from trunk/hind-limb regions of supplementary motor area (SMA) of an unrestrained monkey walking either quadrupedally or bipedally on a treadmill. EMG activity was simultaneously recorded from up to 16 trunk and limb muscles. We found that majority of SMA neurons analyzed (44/51) discharged phasically and/or tonically during the performance of at least one of the two locomotor tasks. Of these locomotor-related cells, more than a half displayed such activity component(s) differently for quadrupedal (Qp) and Bp locomotion, thus the activity of SMA neurons was task-dependent. Interestingly, for Bp locomotion, some cells correlated their activity highly with one of the trunk/hind-limb EMGs (n=7), or peaked during the mid-stance phase (n=15) in a manner similar to hind-limb extensor muscles. Contrary, one third of the locomotor-related cells modulated their activity bi-phasically, which was broadly tuned to the whole step cycle. In addition, small, but significant proportion of the other cells disclosed brief burst activity at the transition of locomotor modes from Qp to Bp during on-going locomotion. Such activity patterns were remote from the recorded EMG activity. Considering input-output organization of SMA, our results suggest that the monkey SMA significantly contributes to the control of locomotion, so as to possibly coordinate movements of trunk and limbs along the rostro-caudal axis as well as movements on the left and right sides of the body.

(COI: No)

P3-123

Physical pain and muscle tonus during hospital bed rest

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Long duration of hospital bed rest induces physical pain in patients. It is believed that pain is correlated with increase in muscle tonus. But data supporting this hypothesis is sparse. The aim of this study is to observe muscle tonus during bed rest using EMG and to evaluate correlations with physical pain. The subjects were 32 healthy adults (M/F = 16/16, mean age 20 ± 1 y/o). EMG were recorded from bilateral eight muscles including M. trapezius, M. erector spinae, M. gluteus medius, M. Obliquus internus abdominis, M. quadriceps femoris, M. biceps femoris, M. tibialis anterior and M. soleus using Neuropack® X1 (NIHON KODEN, Tokyo, Japan). The subjects were instructed to lie in a four-segment adjustable hospital bed for two hours with the head segment elevated to 0° (control), 30°, 45° or 60° (ANGLE). Subjective pain was scored by visual analogue scale (VAS). Vertical and horizontal deviations of the trunk from the original position were recorded. LF/HF ratio of heart rate variability was monitored as indices of sympathovagal balance. Out of 16 muscles observed, seven showed no difference in tonus with time. In nine muscles, EMG activities significantly decreased with time ($p < 0.001$). VAS, LF/HF and deviation of trunk increased with time ($p < 0.001$). ANGLE had significant effect only on vertical deviation of trunk. EMG activity had no correlation with VAS. It is suggested that increase in physical pain due to bed rest does not correlated with increase in muscle tonus.

(COI: No)

P3-124

Axon regeneration and motor function improvement with scaffold-free BMSC sheet transplantation to completely transected spinal cord rat

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In regenerative medicine, the usefulness of cell sheet has been attracting attention. Many studies showed that mesenchymal stem cells (MSC) promote the axonal regeneration of central nervous system, but there is no report of spinal cord injury treatment with sheeted MSC. In this study, we produced bone marrow derived mesenchymal stromal cell sheet (BMSCs) to verify usefulness of the scaffold-free BMSCs transplantation for the completely transected spinal cord model rats. Bone marrow cells were obtained from 7-week-old female F344 rats. BMSCs was made by culture in the standard medium added ascorbic acid. T8 spinal cords of F344 rats were completely transected for 2mm gaps. At 2 and 8 weeks after transplantation, between the two groups: 1)BMSCs transplantation group and 2)control group (gelatin sponge), histological and motor functional evaluation (BBB score) were performed. BBB score of BMSCs group in 8 weeks after injury (mean: 6.75) was higher than the control group. A lot of GAP-43 and Tuj-1 positive axons were observed in the site of sheet transplantation. GFAP strongly positive reactive astrocytes were less than control in the spinal cord stump. By scaffold-free BMSCs transplantation for completely transected spinal cord model rats, axons were regenerated, glial scar formations were inhibited, and motor functions were improved. It was suggested that neurotrophic factor and vascular endothelial growth factor that are secreted by BMSC cause axonal regeneration and glial scar inhibition.

(COI: No)

P3-125

Effect of repeated crush injuries at different intervals on functional recovery of the sciatic nerve

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After a single sciatic nerve crush injury, nerve fibers regenerate and functionalrecovery occurs within 4 weeks in the nerve-crushed adult rats. However, we reported previously that the motor function in the rats with the triple nerve crush injuries every week did not recover to normal range up to 8 weeks because of delay of the reinnervation. In this study, we investigated the effects of repeated nerve crush injuries at different intervals on functional recovery. Double and triple nerve crush injuries of the sciatic nerve were inflicted on adult rats at 1, 2, 3, or 4 weeks intervals. Motor functions were estimated every week until 8 weeks after the last crush injury by the sciatic static index (SSI), a conventional footprint analysis in animal models. SSI is measured by two parameters of 1-5 toe spread and 2-4 toe spread lengths on both sides and widely used for the evaluation of motor function in sciatic nerve-injured rats. At the point of 8 weeks after the last crush injury, reinnervation of the tibialis anterior muscles was estimated by the ratio of β III-tubulin-positive presynaptic nerve terminals for α-bungarotoxin-positive neuroreceptors in the postsynaptic membrane. We report the effects of repeated sciatic nerve crush injuries at different intervals on functional recovery in adult rats.

(COI: No)

P3-126

Architecture of whisker movement related neurons in rat primary motor cortex

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The purposeful movement of biological sensors, such as the motion of the eyes or hands, is an essential part of perception. We don't know what algorithms incorporate movement as part of perception at the level of cortex. Rats move their whiskers to locate and identify objects in their environment. The whiskers provide important tactile information to rats, and therefore have an extended representation in somatosensory and motor cortex. Vibrissa area in primary motor cortex (vM1) controls whisking movements directly via Facial Nucleus (FN) and indirectly via the whisking central pattern generator (CPG) in the brainstem and so on. The facial motor neurons drive the muscles involved in whisking movement. However, what kinds of information from vM1 are sent and where they are sent have not been understood completely. To characterize the motor cortex representation of whisking movements, here we recorded single neuronal activity of vM1 in head-fixed rats performing a behavioral task with juxtacellulary recording method. After recording, we labeled the recorded neuron by injecting plasmid encoding palmitoylation green fluorescent protein (palGFP). We reconstructed and quantitatively analyzed the differences of dendrites and axonal arborization of single vM1 neurons among their firing properties related to whisking movements.

(COI: No)

P3-127

Repetitive masseter muscle activities during NREM sleep in guinea pigs

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Objectives: Rhythmic masticatory muscle activity is observed during NREM sleep in humans. We aimed to physiologically characterize repetitive/rhythmic masseter activities (RMAs) during NREM sleep in guinea pigs in association with cortical and cardiac activities.
Methods: Polygraphic recordings simultaneously with electromyographic (EMG) activity from masseter muscle were made for three hours in the freely-moving guinea pigs. The episodes of the RMAs were visually scored. The electromyographic patterns of RMAs were analyzed quantitatively and compared with those during chewing. Time-course changes of cortical and cardiac activities in relation to the onset of RMAs were also assessed.
Results: RMAs occurred during NREM sleep with a large inter-individual variability in the frequency. Compared to chewing, RMAs was characterized by lower burst activity ($p < 0.001$), longer burst duration ($p < 0.001$) and longer burst intervals ($p < 0.05$). The onset of RMAs was preceded by a decrease in RR-intervals. After the onset of RMAs, the delta power of the electroencephalographic activity and RR interval were transiently changed.
Conclusion: Repetitive/rhythimic patterns of masseter contractions occationally occurred during NREM sleep in the guinea pigs and the occurrence of these episodes were associated with physiological signs of transient arousals.

(COI: No)

P3-128

Tail proprioceptive representation area in the cerebellum identified by using Aldoc-venus knock-in mice

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Aldolase C (Aldoc, also known as zebrin II), a brain type isozyme of the glycolysis pathway, is expressed heterogeneously in subpopulations of cerebellar Purkinje cells that are arranged longitudinally in a complex striped pattern in the cerebellar cortex. Aldoc expression is visualized by expression of a fluorescent protein in Aldoc-Venus knock-in mice. Although Aldoc gene was knocked out in these mice, anatomical features of the brain (size, shape or striped pattern in the cerebellum) and motor coordination (rotarod test) were not different among wild type, heterozygotes or homozygotes. These mice (heterozygotes in particular) enabled in vivo experiments of identified Aldoc stripes in the cerebellum. Although functional localization in vermal lobule VIII has been unclear, we found that Aldoc stripes 1+ and 1- in the caudal apex of this lobuleis intensely innervated by spinocerebellar mossy fiber axons originating from the sacral spinal cord. Flection of the tail produced dynamic spike response in the granular layer in this area, presumably representing mossy fiber activity, in these areas in Aldoc-venus mice. Localized cortical lesioning in this area produced significant decrease in motor coordination in the rotarod test. The results suggest that this area is involved in control of body posture and locomotion by utilizing proprioception of the tail. Kakenhi 25430032.

(COI: No)

P3-129

Glutamatergic plasticity at layers II/III synapses is dependent on the stage of motor learning in rat primary motor cortex

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Synaptic plasticity via AMPA glutamate receptors is associated with memory and learning. To investigate the neuronal mechanism of motor cortical plasticity, we performed a rotor rod test and analyzed layers II/III neurons in the primary motor cortex (M1) using patch clamp method. Motor skill consistently improved within 2 days of training in all animals. In current clamp analysis, 1-day trained rats showed lower, but 2-days trained rats showed the higher resting membrane potential than untrained rats, resulting in a significant increase of firing rate. In voltage clamp analysis, 1-day trained rats exhibited significantly higher AMPA/NMDA ratios and miniature EPSC (mEPSC) amplitude than untrained rats, suggesting an increase in postsynaptic AMPA receptors in the early phase of motor learning. Western blot analysis further indicated a specific phosphorylation of GluA1 subunit of AMPA receptors in 1-day trained rats. On the 2nd day of training, the AMPA/NMDA ratio decreased to the levels in untrained rats. In addition, 2-days trained rats showed the significantly higher mEPSC amplitude and frequency than untrained rats. Moreover, paired-pulse response of EPSC significantly decreased, suggesting the increase in presynaptic glutamate release at the late phase of learning. These results suggest that dynamic changes in the property and glutamatergic plasticity depending on the phase of motor learning in layers II/III neurons in the M1.

(COI: No)

P3-130

Facilitation from the flexor digitorum superficialis to the extensor carpi radialis in humans: a study using a post-stimulus time-histogram method

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Effects of low threshold afferents from musculus (m.) flexor digitorum superficialis (FDS) to m. extensor carpi radialis (ECR) in humans were examined using a post-stimulus time-histogram method with electrical (ES) and mechanical conditioning stimuli (MS) in 4 healthy human subjects. ES to FDS with the intensity below the motor threshold induced excitatory effects (facilitation) in 14/31 ECR motor units in every subject. The remaining motor units received no excitatory or inhibitory effects by ES. The central delay of the facilitation was almost equivalent to that of the homonymous facilitation of ECR. MS to FDS with the intensity below the threshold of the tendon-reflex induced excitatory effects (facilitation) in 31/31 ECR motor units in every subject. The difference between latencies of the facilitation by ES and MS was almost equivalent to that of the homonymous facilitation of FDS by ES and MS. These findings suggest that facilitation from FDS to ECR exists in humans. Group Ia afferents should mediate the facilitation through a monosynaptic path.

(COI: No)

P3-131

Morphological substrates of tectal commissural inhibition and excitation in relation to saccade coordinates and "Listing's law"

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Our previous electrophysiological study showed that excitatory and inhibitory commissural connections existed between the two superior colliculi (SCs). To obtain morphological substrates for these electrophysiological findings, we examined tectal distributions of excitatory and inhibitory commissural neurons (CNs) in the cat SC by injecting tracers into various parts of the SC and double-labelling CNs with GABA and gold particle-conjugated WGA -HRP. Lateral SC injections labeled small GABA-positive CNs in the medial SC and medium-sized GABA-negative CNs in the lateral SC, whereas medial SC injections labeled small GABA-positive CNs in the lateral SC and medium-sized GABA-negative CNs in the medial SC. These morphological results support our electrophysiological findings that mirror-symmetric excitatory pathways link medial to medial upward saccade areas and lateral to lateral downward saccade areas of the SCs, whereas the medial upward saccade area in one SC is inhibited by the lateral downward saccade area in the other SC and vice versa. This pattern of commissural inhibition between two SCs is comparable to that between bilateral vestibular nuclei for the vestibuloocular reflex, indicating that the saccade system uses the semicircular canal coordinates. The excitatory tectotectal commissural connections in bilateral symmetric sites of the rostral SCs might seem to minimize torsional eye movements and contribute to Listing's law.

(COI: No)

P3-132

Plasticity of indirect cortico-motoneuronal excitations in relaxed hand muscles in humans

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We reported that repetitive combined stimulation (RCS) of pyramidal tract and peripheral nerve could induce long-term potentiation (LTP) in indirect cortico-motoneuronal excitations in biceps brachii (BB) of human subjects, which are mediated by cervical propriospinal neurons (PNs). However, the LTP could be induced only when the target muscle was voluntarily contracted, which limits possible clinical use. In animal studies, PNs are known to project various forelimb motoneurons. Because RCS could induce plastic changes in synapses from pyramidal tract to PNs, we hypothesized that the LTP of C-M excitations could also be induced in non-target muscles in upper limb, which are relaxed during RCS. RCS intervention (0.2 Hz, 10 min) was the same as in the previous study. With BB EMG recording under weak contraction, transcranial magnetic stimulation (TMS) to the arm area of left motor cortex (M1) was delivered with right ulnar nerve stimulation. Inter-stimulus interval for the combined stimulation was set at 10 ms, where inputs by both stimuli to reach PNs simultaneously. As previously reported, motor evoked potentials (MEPs) in BB induced by TMS were potentiated after RCS, which lasted for ~65 min. Furthermore, the potentiation could be observed in hand muscles, which showed similar time course to that in BB. These results show that LTP could be induced in muscles without contraction, if RCS induces LTP in another muscle under weak contraction.

(COI: No)

P3-133

Disturbance in information flow through the cortico-basal ganglia pathways in parkinsonian monkeys

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Parkinson's disease (PD) characterized by motor symptoms, such as bradykinesia, rigidity and tremor, is caused by loss of dopaminergic neurons. To elucidate the mechanism causing such symptoms, we examined neuronal activity in the internal pallidum (GPi), the output nucleus of the basal ganglia (BG), of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP, dopaminergic neurotoxin)-treated PD monkeys. The monkeys exhibited moderate to severe motor symptoms. Motor cortical stimulation induced triphasic responses composed of early excitation (Ex) followed by inhibition (Inh) and late Ex in the GPi of normal monkeys. After MPTP treatment, however, cortically evoked Inh was mostly lost with little changes in spontaneous firing rates. Intravenous L-dopa injection alleviated motor symptoms and restored cortically evoked Inh in the GPi. Blockade of subthalamic nucleus (STN) by muscimol injection improved bradykinesia, suppressed cortically evoked Ex, and restored cortically evoked Inh in the GPi. These results suggest that disturbance in information flow through the cortico-BG pathways is responsible for PD symptoms. Under normal conditions, signals through the cortico-striato-GPi *direct* pathway induce Inh in the GPi and properly release motor actions by disinhibiting the thalamus. On the other hand, in PD, signals through the *direct* pathway induce diminished Inh in the GPi and fail to disinhibit the thalamus, resulting in bradykinesia.

(COI: No)

P3-134

Synaptic inputs underlying motoneuronal firing during locomotion in the α-chimaerin knockout mouse spinal cord in vitro

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For the formation of the motor circuit in the developing mammalian spinal cord, interaction between tyrosine kinase receptor EphA4 and its ligand ephrinB3 is crucial, regulating the projection of a subpopulation of ipsilaterally projecting neurons. The interaction of these two molecules functions as a barrier preventing ipsilaterally projecting EphA4+ axons from crossing the midline. Deletion of the EphA4 or its downstream-signaling molecule alpha-chimaerin causes aberrant midline-crossing of these ipsilateral-projecting axons in the spinal cord and leads to a hopping gait in mice. In this study we examined how lumbar motoneurons (MNs) are modulated by excitatory and inhibitory synaptic inputs during locomotor-like rhythmic activity in the isolated spinal cord preparation taken from alpha-chimaerin knockout (Chn1-KO) neonates. Similar to wildtype MNs, firing of an individual Chn1-KO L2-flexor-related-MN was time-locked with the ipsilateral flexor activity but not with that of the contralateral side indicating that it is unlikely that the firing pattern of Chn1-KO MNs is shaped by direct synaptic inputs from the contralateral network. Furthermore, we estimated the instantaneous frequency of excitatory and inhibitory synaptic inputs during the rhythmic activity from the membrane voltage trace based on the Ornstein-Uhlenbeck model. Preliminary results suggest that during locomotion, Chn1-KO MNs are synaptically modulated in a similar way to wildtype MNs.

(COI: No)

P3-135

The effect of cyclic illusory movement on corticospinal excitability of muscles in the contralateral limb

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When human move their wrist periodically, the neural activities in the contralateral forearm muscles are modulated so as to correspond to the phase of the wrist movement. It is considered that such neural modulation in remote limb enhances synchronization in bimanual movements. The detailed neural mechanisms are still hardly known. In order to investigate the effect of sensory process on neural modulation, we tested whether just illusion of rhythmic movement of one hand modulates corticospinal activity in the contralateral forearm muscle depending on the phase of illusory movement. The experimental tasks were 1) voluntary movement of right hand, 2) kinesthetic illusion induced by tendon vibration (80Hz), 3) no illusion with vibration, 4) passive movement. During these tasks, transcranial magnetic stimulation of the left motor cortex was delivered at random timing, and then motor evoked potentials (MEPs) were recorded from extensor and flexor of resting right wrist. Kinesthetic illusion of the left wrist without overt movement or muscle activity modulated the MEP amplitude in the contralateral forearm muscles depending on illusory movement phase just as observed during voluntary movement. These results indicate that only perception of kinesthetic movement can produce the MEP modulation of resting limb depending on movement phase of contralateral limb. Thus, sensory process would be important in the functional connectivity between bimanual hands.

(COI: No)

P3-136

Convergence of multi-pathway signals in single cerebellar granules cells in vivo

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Somatosensory signals from the facial area are conveyed to the cerebellar cortex directly via trigeminocerebellar pathway as well as indirectly via cortico-ponto-cerebellar pathway. Both of these pathways, forming mossy fibres, project to the granule cells in the cerebellar cortex. It was not known whether these pathways project to different populations of granule cells or converge onto the same granule cells. To address this issue, we made whole-cell patch-clamp recordings from single granule cells in the crus II area of anaesthetized mice. In these experiments, knock-in mice expressing Venus fluorescent protein by aldolase C promoter were used to visually identify the sagittal zones. When the upper lip was stimulated by air puff, excitatory postsynaptic currents (EPSCs) were evoked in granule cells in the aldolase C compartments 5+ and 5-. A majority (4/6) of the responding cells had two components of EPSCs. The latency (time from the onset of stimulation to the peak) of fast and slow components were 6.3 ms and 25.9 ms (n = 4), respectively, while the field potentials simultaneously recorded from the cerebral sensory cortex had a single component with intermediate latency (19.6 ms), suggesting that the fast and slow components in granule cells were the direct trigeminal input and the indirect pontine input, respectively. These results suggest that a substantial population of paravermal granules cells receive convergent inputs from direct and indirect pathways.

(COI: No)

P3-137

Anatomically structured burst spiking in the thalamic reticular nucleus: implications for a novel functional organization of thalamocortical loop circuitries along the rostrocaudal neural axis

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Thalamic reticular nucleus (TRN), a cluster of GABAergic cells projecting to thalamic nuclei, occupies a highly strategic position to regulate information processing in the thalamocortical loop. TRN cell activity is characterized by burst spiking that imposes significant influences on thalamic and thereby cortical cell activities. In view of the previous findings that the intensities of burst spiking in spontaneous activities of visual and auditory cells in the caudal TRN are graded in a similar manner along the rostrocaudal neural axis, the present study was carried out to address the question of whether the gradient of burst spiking extends in the whole rostrocaudal range of the TRN. Recordings of spontaneous activity were obtained from 49 cells in four anesthetized rats. Cells were labeled with neurobiotin to determine locations. The number and averaged inter-spike interval of spikes in a burst became larger and shorter as cells were located more rostrally in the putative domain that contains visual, auditory and somatosensory cells. This gradient, however, appeared to be reversed in the rostral end of the TRN that operates for motor and limbic functions. The results suggest that burst spiking is anatomically structured along the rostocaudal neural axis in the whole sensory domain of the TRN. There could be a novel functional organization that allows the TRN to impose graded influences on sensory processing in the thalamocortical loop across sensory modalities.

(COI: No)

P3-138

Visual short-term memory dependent on the diversity of protocadherin-α cluster in mice

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The clustered protocadherins (Pcdh) are assumed to play important roles for the formation of sophisticated neural networks. Of the 12 clusters in cPcdh-α, cPcdh-α 1, 12 mice have only cPcdh-α 1 and α 12, so that cPcdh-α diversity is largely reduced. To investigate the roles of clustered structure of cPcdh-α in higher brain function, we investigated visual short-term memory. First, we tested short-term memory of spatial information using a T-shaped maze task. The performance of cPcdh-α 1, 12 mice was significantly worse than that of wild-type mice. We further tested visual short-term memory of shape information. We developed an M-shaped maze equipped with a display. In a control task, a cue shape was presented at center of the display, and the two choices including the original cue were presented at both branches with overlapped timing. Mice must select the cue, or the other in other experiments, to get reward. In the short-term memory task, the delay period was set at 20 s between the presentation of the cue and two choices. The performance in cPcdh-α 1, 12 mice was significantly worse than that in wild-type mice. After this visual short-term memory sessions were finished, we confirmed that mice could choose a pair of alphabets, which they had never seen, based on the short-term memory. These results indicate that mice can memorize shapes as complex as alphabets, and the diversity of Pcdh-α is required for the visual short-term memory.

(COI: No)

P3-139

Different members of face-responsive neurons in monkey area TE contribute to global categorization of faces and to upright-face versus inverted-face categorization

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We have reported that face-responsive neurons in monkey area TE represent information about a global category, i.e. human vs. monkey vs. shapes earlier than information about more detailed categories, e.g. facial expression. To examine whether neurons respond to inverted faces showing similar characteristics to upright faces on a face-by-face basis, we analyzed activities of 119 face-responsive neurons in area TE of two rhesus monkeys (Macaca mulatta), performing a fixation task. Test stimuli were colored pictures of monkey faces (4 models with 4 expressions), human faces (3 models with 4 expressions), geometric shapes, and inverted pictures of the faces. Population vectors consisting of responses for each stimulus were computed in a window 115-165 ms after a stimulus onset. Sparse logistic regression was applied individually to the vectors for the upright monkey vs. human faces (GL), for the upright vs. inverted human faces (HUI), and for the upright vs. inverted monkey faces (MUI). The number of neurons contributed to GL, HUI, and MUI was 2, 4, and 5, respectively. Three neurons were found in common for HUI and MUI. No neuron and one neuron was found in common for GL and HUI and for GL and MUI, respectively. The results suggest that different members of the neuronal population contribute to GL and to HUI or MUI, but that HUI and MUI have contributing members in common. Supported by Grants-in-Aid for Scientific Research on Innovative Areas "Sparse modeling" (26120535).

(COI: No)

P3-140

Representation of binocular depth in macaque visual area MT proved with varied temporal frequencies of visual stimuli

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Binocular disparity is a cue for binocular stereopsis. The visual system has correlation-based and match-based representations for binocular disparity. These representations are characterized by disparity tuning functions to anti-correlated RDSs (aRDSs). In aRDSs, the luminance contrasts of dots are reversed in one eye. The correlation-based representation should have inverted tuning functions for aRDSs relative to those for normal, correlated RDSs; the match-based representation should lose disparity selectivity for aRDSs. Human psychophysical experiments suggest that stimulus temporal frequency changes the relative contributions of the two representations to stereopsis. Here, we hypothesized that the temporal frequency alters the neural representation of binocular disparity in the visual cortex. To test this hypothesis, we recorded responses from 41 disparity-selective neurons in macaque middle temporal area (MT). The disparity-tuning curves of these neurons were tested both with normal and anti-correlated RDSs and with slow and fast pattern refresh rates of the RDSs. We fitted Gabor functions to the tuning curves to estimate the tuning-curve shape. For both slow and fast refresh rates, the tuning curves inverted the shapes when binocular anti-correlation was applied to RDSs. Our results indicate that temporal frequency does not alter the correlation-based representation of disparity in area MT.

(COI: No)

P3-141

Superior colliculular neurons are involved in detection of face-like patterns in monkeys

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Facial recognition plays an important role in social communication in primates. Previous neurophysiological and imaging studies suggest that the superior colliculus (SC) is implicated in social behaviors and facial information processing. However, specificity of SC neuronal responses to facial stimuli remains unclear. In this study, we recorded monkey SC neuronal activities during discrimination of various monochrome face-like and non-face-like patterns in a delayed non-matching to sample (DNMS) task. Each pattern consisted of one of 4 face contours (rice scoop, star, circle, square) and 5 facial features (2 eyes, 2 eyebrows, 1 mouth). Each non-face-like pattern consisted of the same facial contours, but the facial features were random positioned in the facial contours, or included no facial features. Of 405 SC neurons recorded, 138 neurons responded to visual stimuli. Of these, 116 neurons were tested with the all stimuli. The results showed that SC neurons responded stronger and faster to upright and inverted face-like patterns than to non-face-like patterns. Furthermore, response latencies were shorter in SC neurons with upper receptive fields than those with lower receptive fields. Mean response latencies were also shorter in SC neurons in the superficial layers than in the deep layers. Furthermore, response magnitudes to original images were significantly correlated to those to white/black reverse images in about 50% of SC neurons. These results provide evidence for SC involvement in detection of face-like patterns.

(COI: No)

P3-142

The similarity of receptive field properties in connections between the retina, the lateral geniculate nucleus and the primary visual cortex of the cat

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In the early visual system, receptive field (RF) properties, such as orientation selectivity and spatial frequency (SF) selectivity, are key features of visual information processing. Although many studies have investigated retinal, geniculate, and cortical functions, relatively few studies have been conducted with a particular focus on the similarity/difference of the functional properties among the regions. In this study, we simultaneously recorded multi-unit activity from the retino-geniculate and geniculo-cortical neural populations from anesthetized cats. Then, we compared the RF properties obtained using the reverse correlation method with noise or flashing grating stimuli between the populations with or without the significant functional connections. We found that, regardless of the connections, preferred SF became low to high through the pathway, which might reflect the converging retino-geniculate projections and the narrowly tuned geniculo-cortical projections. Also we found that the populations with the functional connections sharing the similar preferred orientations, suggesting that there were the connections depending on the preferred orientations through retina to V1.

(COI: No)

P3-143

Interaction between neuronal responses to multi-site microstimulation in the mouse visual cortices in vivo

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It has long been suggested that cortical microstimulation is feasible for artificially restoring a certain level of auditory, tactile, or visual sensation, by directly exciting the target neurons in the cerebrum. In such a cortical neuronal prosthesis, it is required to deliver electrical stimuli to multiple sites in accordance with the topological map, regardless of their sensory modalities. However, little has been known about interactions of the neuronal responses induced with multiple electrodes. In the present, we examined the spatio-temporal neuronal responses to multi-site microstimulations in the mouse visual cortices in vivo using the voltage-sensitive dye imaging technique. When electrical pulse stimuli were delivered simultaneously from spatially separated two electrodes, depolarizing responses were evoked in the corresponding regions in the primary, and then secondary visual cortical areas (V1 and V2). These responses were smaller in amplitude as well as in spatial extents in both V1 and V2 compared with a linear summation of two responses that are evoked independently with each of the electrodes. Such a suppressive interaction became less significant as the stimulation interval between the two electrodes was increased from 10 to 100 msec. Similar results were obtained when the stimuli were delivered from three electrodes. The nonlinear interaction among responses induced with multi-site stimulations should be taken into account for designing effective stimuli in cortical neural prostheses.

(COI: No)

P3-144

Postnatal developmental observations of abnormal retinal terminal aggregation in the cPcdh-α KO mice

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Clustered protocadherins (cPcdhs) belong to the cadherin superfamily and are playing important roles on the cellular diversity and interaction at neuronal surface in the mammalian central nervous system (CNS). cPcdhs consist of three families, cPcdhs-α, -β and -γ, and each family shows distinct spatial and temporal distribution in the CNS. Their roles in the establishment of nervous system, however, are still unclear. To investigate the functional features of cPcdhs-α during postnatal development, we produced cPcdhs-α deleted mice. They could grow up without gross abnormality, but we found aberrant terminal distribution of retinal axons in the dorsal lateral geniculate nucleus (LGd), the primary relay center of the visual system. There were many strange huge aggregations of retinal terminals, as large as neuronal soma size, within the LGd. In the postnatal developmental study, we found that the huge aggregations firstly appeared during P10−P14, just before eye opening. The retinal terminals of the earlier stages of postnatal development were not apparently different from those of the wild mice. This suggests that cPcdhs-α might play important roles in the refinement stage which starts around the eye opening, following the completion of eye-specific segregation stage of retinal terminals in the LGd.

(COI: No)

P3-145

Transporter-independent choline uptake in the mouse retina

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Choline, an essential precursor for acetylcholine synthesis, is transported into synaptic terminals through high affinity choline transporter (hCT) in cholinergic neurons. We found that the immunoreactivity for hCT of the ON-cholinergic amacrine cells (ON-CACs) was significantly stronger than the immunoreactivity for hCT of the OFF-CACs in the mouse retina. We have previously reported that P2X2-purinoceptors, which are permeable to large cations, are specifically located in OFF-CACs. In this study, therefore, we examined whether the less accumulation of hCT in the OFF-CACs is compensated by choline uptake through P2X2-purinoceptors. When ATP was applied to P2X2-purinoceptor expressing HEK293 cells, inward current was detected even when the extracellular Na^+ were replaced with equimolar $choline^+$. The permeability to $choline^+$ was also found in the OFF-CACs in the mouse retina. Choline current was activated by an application of ATP-γ-S but not by α, β-methylene ATP or benzoyl-benzoyl-ATP. In the presence of pyridoxalphosphate-6-azophenyl-2', 4', -sulfonic acid, an application of ATP did not induce any choline current. Furthermore, cholinergic current was increased when extracellular Ca^{2+} concentration was reduced. These physiological and pharmacological characteristics support the notion that P2X2-purinoceptors permeate choline, and that cholinergic transport mechanism is different between ON-CACs and OFF-CACs. The P2X2-purinoceptors might work as an alternative pathway of choline transport especially in the OFF-CACs of the mouse retina.

(COI: No)

P3-146

The dry eye enhances cold cell sensitivity to capsaicin

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Previous studies have found that cold cells innervating the cornea are sensitive to the ocular fluid status of the corneal surface and may be responsible for the regulation of basal tearing. In addition, we have shown that an experimental dry eye condition modifies the thermal and menthol responses in these corneal primary afferent neurons. In the present study, we examined the effect of dry eye on the sensitivity of cold cells to the TRPV1 agonist capsaicin. Unilateral dry eye condition was created by excision of the left exorbital and infraorbital lacrimal glands. Extracellular, single-unit recordings were performed in anesthetized animals 1 week after gland excision and in age matched controls. Electrodes positioned in the trigeminal ganglion were used to isolate and characterize cold-sensitive neurons. Responses to thermal stimulation were examined 5 min after the application of capsaicin (3 nM- 3 uM). At low concentrations (<300 nM), capsaicin did not affect the rate of ongoing and cold-evoked activity in both groups of animals. High concentrations of capsaicin (>= 300 nM) suppressed the ongoing and cold-evoked activity in both groups of animals, overall, capsaicin induced greater suppression in dry eye animals. We applied TRPV1 antagonist, capsazepine 30 min before capsaicin application. Capsazepine blocked the capsaicin-induced suppression of cold cell activity. These results indicate that dry eye sensitizes cold cells to capsaicin mediated by TRPV1 channels.

(COI: No)

P3-147

Phagocytic ability of Müller glia in the damaged retina

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It is well established that microglia, the resident phagocytes in the central nervous system, play a major role in the clearance of dead cells after neuronal damage. Microglia are also present in the retina and have been considered as the principal phagocytes in the retina that are recruited to the site of damage and remove dead neurons. However, we have recently observed that, in the rat model of photoreceptor damage, dead photoreceptors were removed from the outer nuclear layer immediately after induction of photoreceptor death and replaced by proliferating Müller glia. This suggests that Müller glia may repair damage by removing dead photoreceptors by phagocytosis. To test this possibility, we examined the phagocytic ability of Müller glia in the rat retina after methyl nitrosourea-induced photoreceptor damage. Double immunofluorescence for glutamine synthetase, the Müller glia marker, and rhodopsin, the rod photoreceptor marker, revealed rhodopsin-positive photoreceptor debris within the cytoplasm of Müller glia, consistent with the possibility that Müller glia are able to phagocytose dead photoreceptors. On the other hand, few microglia were detected in the ONL during the stage of photoreceptor removal. These results indicate that Müller glia have a previously unrecognized role as phagocytes that remove dead neurons after retinal damage.

(COI: No)

P3-148

Differentiation of gap-junctionally connected amacrine cells and modulation of channel opening of their electrical synapses

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Electrical synapses are present in retinal neurons expressing channel subunit, connexins (J Neurosci, 2004; Brain Res, 2012). Electrical current spread through connections of cells is expected to modulate chemical synapses. Our studies revealed channel opening of gap junctions between several types of retinal amacrine cells is regulated by intracellular cyclic AMP as well as intracellular Ca^{2+} concentration (Brain Res, 2012). Individual amacrine cells show specific coupling patterns (J Intgra Neurosci, 2005). Based on coupling patterns and dendritic morphology, six different classes of homotypic lateral connections between amacrine cells of the same subtype were identified. Two types of cell-specific dendritic contacts, tip-contacts and cross-contacts, were found. Electrical synapses between tip-contact cells are regulated by intracellular cyclic AMP. I investigated synaptic contacts of these amacrine cells by electron microscopy as well as laser scanning confocal microscopy. Tip-contact cells make output synapses only onto axon terminals of retinal bipolar cells. Whereas cross-contact cells make conventional synapses onto dendrites of retinal ganglion cells. I investigated channel opening of electrical synapses between cross-contact cells under dual whole-cell patch clamp recordings. Gap junctions between cross-contact cells closed under intracellular high Ca^{2+} concentration (>300nM), but did not close under intracellular application of cyclic AMP. These results suggest that cell-specific electrical synapses play important roles in differential synapses from retinal amacrine cells.

(COI: No)

P3-149

Mechanisms of the retinotopic map reorganization induced by monocular enucleation

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Visual input is received by retinal ganglion cells and their axons project mainly to the lateral geniculate nucleus (LGN) of the thalamus. Then neurons in the LGN send their axons to the primary visual cortex (V1). In this visual pathway, the retinogeniculate and geniculocortical connections are organized to form an accurate retinotopic map during the developing stage. Both molecular guidance and neural activity are thought to play an important role in the formation of the topographical arrangement, although the precise mechanism is still unknown.

A previous electrophysiological study using hamsters reported that monocular enucleation in early postnatal days induces a disarrangement of the retinotopic map in V1, resulting in the duplication of the central visual field. We used an optical imaging technique to investigate the change of the retinotopic map more precisely in the monocularly enucleated (ME) animals. We observed the duplication of the retinotopic map clearly across V1 which is ipsilateral to the intact eye. The responding region to the ipsilateral eye in V1 of the ME animals was larger compared with that of normal animals. This functional change may reflect a reorganization of the neural connections. We demonstrate possible anatomical correlates of the map duplication and the influence on the expression of guidance molecules which affect axon growth.

(COI: No)

P3-150

Relationship between barometric hypersensitivity and autism - morphological analysis of the autism model rat

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Autism spectrum disorder (ASD) is one of the developmental disorders according to three core features of social deficits, communication impairments, and repetitive or stereotyped behaviors. In addition to these features, hypersensitivity is often appended to the symptom in ASD. It has been reported that the receptor organ of barometric pressure locates in inner ear, but its mechanisms has not been known. Previously, we reported the autistic rat model through prenatal thalidomide exposure and its abnormal development of serotonergic neuron in the brain with morphological and behavior analyses. In this study, we examined morphological analyses of inner ear in the autistic rat model. Pregnant Wistar rat were exposed to thalidomide on embryonic day 9. Each offspring was perfused with paraformaldehyde and inner ears were dissected out. Immunohistochemical analyses of frozen sections in the inner ear and surface preparation of organ of Corti were performed. Histological differences between thalidomide-exposed rat and control could not be found about inner and outer hair cells in the organ of Corti and these synapse formation. These results suggest that the inner ear in the autistic model rat is morphologically normal.

(COI: No)

P3-151

Local Na^+ current forms the unique electrical property of the epithelial tissue essential for the endocochlear potential in the inner ear

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Cochlear endolymph exhibits a potential of +80 mV relative to an ordinary extracellular solution, perilymph. This called endochclear potential (EP) is essential for hearing. The EP is maintained by the lateral cochlear wall that comprises outer and inner epithelial layers. The basolateral surface of the former is exposed to the perilymph, whereas the apical surface of the latter face the endolymph. Between the two layers lies the extracellular compartment that shows a highly positive potential. This interlayer potential (ILP) is a source of the EP and governed by a K^+-diffusion potential that depends on a large K^+-gradient across the apical surface of the outer layer. Our electrophysiological experiments and computational model revealed that the gradient is controlled by the unidirectional K^+-transport across the lateral wall. We previously found that the outer layer is continuously depolarized of +7 mV relative to the perilymph. Although this unique property is indispensable for the positive ILP, how it is formed remains uncertain. The theoretical approaches based on our former model predicted that Na^+ current depolarizes the basolateral surface of the outer layer. In support of this, perilymphatic perfusion of low [Na^+] solution hyperpolarized the outer layer and reduced the ILP and EP. Thus, the Na^+ current is a critical regulator for the EP.

(COI: No)

P3-152

Proteomic analysis of the epithelial tissue that drives membrane transport systems in the cochlea of the inner ear

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Cochlear endolymph in the inner ear exhibits 150 mM [K^+] and a positive potential of +80 mV. These properties are essential for hearing and maintained by the K+-transport across the epithelial tissue, stria vascularis. Majority of the proteins involved in the K^+-transport have been identified. The stria vascularis also drives a variety of other membrane transports to function the cochlea. To clarify the proteins underlying these transport systems, we analyzed the membrane fractions of the stria by a mass spectrometry. We identified 1,664 membrane proteins, which contained 25 ion channels and 79 transporters. 16 of the former and 65 of the latter have been for the first time detected in the stria. Network analysis suggested that Ca^{2+} signaling would play pivotal roles in the strial transport. Of interest, we identified 20 candidates for uncloned deafness genes. Our protein library is useful to elucidate not only molecular architecture of the membrane transport systems in the stria but also pathological processes of hearing disorders.

(COI: No)

P3-153

Evidences of the K^+-circulation current that controls the electrochemical properties in the cochlea of the inner ear

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Cochlear endolymph exhibits a high [K^+] of 150 mM and a highly positive potential of +80 mV. We previously revealed by electrophysiological assays and a computational model that the lateral cochlear wall maintains these unique properties with the K^+-circulation current which flows between the perilymph and the endolymph. Its dysfunction causes deafness. The lateral wall consists of two epithelial layers; the inner and the outer layers. The latter expresses Na^+, K^+-ATPases on its perilymphatic surface. However, it remains uncertain whether they contribute to the circulation current. An inhibition of these ATPases decreased the intracellular [K^+] of the outer layer and consequently impaired the endolymphatic potential. Based on this experimental data, in this study we renewed the computational model, where the K^+-circulation current set to flow through the ATPase in the outer layer. The model predicted that the block of the ATPase reduced the circulation current, which leaded to a decrease of the extracellular [K^+] between the two layers. Indeed, this alternation could be observed by *in vivo* electrophysiological experiments. These results support the concept of our model that the K^+-circulation current occurs across the lateral wall and establishes the endolymphatic properties.

(COI: No)

P3-154

5-HT3 receptor expression in the mouse vestibular ganglion

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Introduction: 5-HT3 receptor is a ligand-gated ion channel. Previous studies have shown 5-HT3 receptor expression in various neural cells of the central and peripheral nervous systems. Although the function and distribution of the 5-HT3 receptor has been well established, no study has yet determined its localization and function in the peripheral vestibular nervous system. To address this question, we investigate here the localization of the 5-HT3 receptor in the mouse peripheral vestibular nervous system.
Methods: C57BL/6J wild-type (WT) and 5-HT3 receptor knock-out (KO) mice were used in this study. We performed RT-PCR and in situ hybridization to examined 5-HT3 receptor mRNA localization in the inner ear. Moreover, we studied the physiological effects of a selective 5-HT3 receptor agonist (SR57227A) on freshly isolated VG neural cells from adult mouse using the measurement of intracellular calcium ion concentration (Ca2+ imaging).
Results: We found that both 5-HT3A and 5-HT3B receptor mRNA is expressed in VG neurons and also that 5-HT3 receptor mRNA is localized in the VG of the inner ear. 5-HT3A receptor mRNA is expressed in approximately 30% of VG neurons, while 5-HT3B receptor mRNA in VG neurons is expressed with a much lower signal. SR57227A induced increases in intracellular calcium in several VG cells from WT mice. However, SR57227A caused no change in VG neurons from 5-HT3A receptor KO mice.
Conclusions: These findings suggest that functional 5-HT3 receptors are synthesized in VG neurons and might modulate the peripheral vestibular nervous system.

(COI: No)

P3-155

Brain responses induced by odor and odorless air stimulation in human: 7 Tesla fMRI study

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It has been demonstrated that olfactory sensory neurons respond to both odor and mechanical stimuli. However, processing mechanism of different sensory information received by olfactory sensory neurons remains unclear. To clarify the brain area related to odor and mechanical sensory processing, we investigated brain responses induced by odor and mechanical stimuli application to nostrils with fMRI. BOLD-signal changes evoked by odor stimuli are known to comparatively small. Thus, we used ultra high field (7 Tesla) MRI to increase BOLD signal. Odorant stimulation (isovaleric acid, peppermint and coffee odor) and odorless air (mechanical) stimulation were applied to nose by air pressure. Activation by odor and mechanical stimuli were detected in piriform cortex, amygdalae, hippocampus, thalamus, cingulated cortex, insula, orbitofrontal cortex and somatosensory cortex. In the piriform cortex, odor stimuli induced activation at anterior and mechanical stimuli induced activation at posterior. Mechanical stimuli induced strong activation in thalamus. In the insula, olfactory stimuli evoked activation in the anteroventral portion and mechanical stimuli evoked activation in the posterodorsal portion. These results suggested that odor and mechanical information from olfactory epithelial cells project different brain area.

(COI: No)

P3-156

Responses of mature and immature rats to P-mix derived from wolf urine

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Urine excreted from common grey wolf (Canis lupus) has been used as a repellent for various kinds of mammals. Previous our studies showed that pyrazine analogues contained in wolf urine (P-mix) induced avoidance and fear behaviours in mice (Osada et al., 2013) and Hokkaido deer (Osada et al., 2013). Mice and Hokkaido deer have not any experiences that they have threated by wolves during living periods because wolves died before 100 years ago in Japan. This suggests that P-mix induces innate fear in these animals. In the present study, first, we addressed question that P-mix induces a fear-related behaviour and avoidance in mature rats. Then, we examined effects of P-mix on immature rats to explore whether P-mix induces innate fear responses in immature rats. P-mix also induce avoidance in immature rats. Previous study also showed that P-mix induced excitation of neurons at the accessory olfactory bulb (AOB), which receives pheromonal information. Exposure of mature and immature rats to P-mix induces expression of Fos-ir cells, which are excited neurons, at the AOB of mature and immature rats. The present results showed that P-mix induces fear-related responses in immature and mature rats via the AOB.

(COI: No)

P3-157

Properties of olfaction in patients with panic disorder

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Panic disorder (PD) is classified in V. Anxiety disorder of DSM-5, which is characterized by sudden bursts of panic attacks accompanied by heart palpitations, sweating, dyspnea, and abnormal sense. Patients often suffer from severe terror and discomfort reaching the peak in a few minutes. Although anti-depressants (SSRI) or cognitive behavior therapy are applied, it is very difficult to recover completely. It is reported that olfactory function of PD patients is normal, using UPSIT, as well as olfactory-triggered panic attacks experienced in 45 % of patients. Taken together, we examined olfactory function of PD patients by T&T olfactometry and Open Essence, after answering to the questionnaire on self-evaluation. Eight patients of PD participated in this study. All of them have anti-depressant or antianxiety drugs administered. Unfortunately they still suffer from panic attacks at frequency of once per one to 6 months. PD patients has hypersensitive olfactory function, although most of them are unaware of it. These results suggest that approach to olfaction of PD patients or odor administration to them may be much useful in diagnoses or treatment of PD. This study was approved by Kochi Medical School Clinical Research Center (ERB-000397), and we obtained informed consent from all subjects before study participation.

(COI: No)

P3-158

Expression of G proteins in the olfactory organs of *Pelodiscus sinensis*

Nakamuta, Shoko; Yamamoto, Yoshio; Nakamuta, Nobuaki (*Iwate Univ., Iwate, Japan*)

Many vertebrates have two olfactory organs, the olfactory epithelium (OE) and the vomeronasal organ (VNO). Since fish and aquatic mammals do not have the VNO, presence of the VNO is thought to be closely related to the adaptation for terrestrial life. In this study, the olfactory organs of a semi-aquatic turtle, *Pelodiscus sinensis*, were investigated to examine their adaptation for the aquatic and terrestrial environment. The nasal cavity was largely divided into the upper and lower chambers. They were lined by sensory epithelia, which can be regarded as the OE and the VNO, respectively. The upper chamber epithelium was supposed to detect odorants in the air and the lower chamber epithelium in the water, since associated glands were found only in the upper chamber epithelium. Immunoreactivity for Golf, a member of G proteins coupled to the odorant receptors, were detected in the cilia on the free border of epithelia lining both upper and lower chambers. In addition, the Golf-positive cilia in the upper chamber epithelium were longer and more intensely labeled than those in the lower chamber epithelium. These results suggest, although differences in the cell shape are exist, that receptor cells positive for Golf are distributed both in the OE and the VNO of *P. sinensis*, and involved in the detection for odorants both in the water and air. It is interesting to note that the same characteristics are found in other semi-aquatic turtles, *Geoclemys reevesii* and *Trachemys scripta elegans*, both of which belongs to the other family than the *P. sinensis*, considering their adaptation for the aquatic and terrestrial environment.

(COI: No)

P3-159

Effects of external Cl^- on the electroolfactogram recorded from the goldfish olfactory epithelium submerged in saline solutions

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Most olfactory receptor neurons (ORNs) of vertebrates bear cilia or microvilli at their dendrite tips. The ciliated ORNs (cORNs) have a signal transduction using adenylate cyclase. The Ca^{2+} influx through cAMP-gated channels opens Ca^{2+}-activated Cl^- channels, which amplify the response. While the microvillus ORNs (mORNs) of many terrestrial vertebrates are mostly located in the vomeronasal system, teleosts have both of cORNs and mORNs in the same olfactory epithelium, allowing us to compare them in the same epithelium. Although mORNs appear to utilize Phospholipase C to induce the olfactory response, the role of C^-l in the fish mORNs are not clear. We tested the effect of the external Cl^- on the electroolfactogram (EOG) of goldfish. The nose tissue was isolated from the body and submerged in the Ringer solution for EOG. The $[Cl^-]$ of the solution was reduced from 126.5 to 4.5 mM just after the end of 1-s application of 250 μM IBMX that raises [cAMP] in cORNs. In this condition, the IBMX response was enhanced by 21 ± 14% (SD, n=4) compared to the control response recorded without the Cl^- reduction, suggesting the contribution of the Cl^- efflux in the IBMX response. When the olfactory epithelium was stimulated by 1 mM serine (0.5 s), the Cl^- removal did not induce the detectable increase of the EOG response; 4 ± 3%, n=8. Serine response may occur mainly in mORNs, and the Cl^- efflux may not produce a detectable contribution to the response of goldfish mORNs in our recording condition.

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(COI: No)

P3-160

Whole-cell recording from goldfish olfactory receptor cells in the slice preparation of the olfactory epithelium

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The olfactory epithelium (OE) of teleost fish has different types of olfactory receptor neurons (ORNs), including ciliated ORNs (cORNs) and microvillus ORNs (mORNs), which have been expected to have different signal transduction cascades involving adenylate cyclase and phospholipase C, respectively. Teleosts have no vomeronasal organ (VNO). Both of the cORNs and the mORNs of teleosts are distributed in the same OE, whereas cORNs and mORNs of many terrestrial vertebrates almost exclusively localize in main OE and VNO, respectively. Thus, the teleost OE is a good platform to compare the cell properties of cORNs and mORNs in a same preparation of OE. In the present study, we developed a slice preparation of goldfish olfactory epithelium to record olfactory responses by using whole-cell patch clamp technique. The olfactory organ (rosette) was dissected from the head of goldfish in a 0.5 mM-$Ca2^+$ saline solution. Several lamellae of the olfactory rosette were separated from the tissue, and laid flat on a piece of a nitrocellulose filter membrane (3×8 mm) coated with a cyanoacrylate adhesive. The flat-mount lamellae with the filter membrane were cut into 250 μm slices by a razor-blade tissue chopper slicer. The current responses of goldfish ORNs to IBMX, a bile acid and/or amino acids were successfully recorded with the obtained sliced tissue of goldfish OE. This work was supported in part by KAKENHI (22500351).

(COI: No)

P3-161

5T4 oncofetal trophoblast glycoprotein regulates the sensory experience-dependent dendritic development in newborn olfactory bulb interneurons

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Sensory input regulates the development of various brain structures, including the retina, cortex and olfactory bulb (OB). Little is known about how sensory experience regulates the dendritic development of OB interneurons, such as granule cells (GCs). Recently, we identified, with DNA microarray and *in situ* hybridization screenings, an oncofetal trophoblast glycoprotein *5T4* gene, whose expression in the OB interneurons is dependent on sensory experience (Yoshihara *et al*, J Neurosci 32, 2217, 2012). In this study, we characterized 5T4-knockout mice to know its physiological role in the dendritic development of OB GCs. *5T4*-knockout mice resulted in a significant reduction in the dendritic branching of OB GCs, while *5T4* overexpression could rescue the reduction of the dendritic arborization in its knockout GCs. Then, we conducted behavior tests for *5T4*-knockout mice. Interestingly, *5T4*-knockout mice were less sensitive in odor detection than the wild-type mice, and impaired the acquisition of the two-related-odor discrimination task, although they possessed the olfactory detection ability in the food seeking task. Taking account of electrophysiological data for external tufted cells from *5T4*-knockout mice, these results demonstrate that 5T4 contributes to regulate the sensory experience-dependent dendritic development of interneurons and the formation of functional neural circuitry in the OB.

(COI: No)

P3-162

ER stress induced in the OB inhibits olfactory learning

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It is well known that the endoplasmic reticulum (ER) stress links to neuronal death in various neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease. It is also reported that most patients complain impairment of olfactory recognition. Therefore we examined if ER stress provoked by tunicamycin (TM) infusion has an inhibitory effect on synaptic plasticity in the olfactory bulb (OB) underlying aversive olfactory learning. Without vision, young rats can learn their mother's odor and approach her. They do this in part by learning their mother's odor as a conditioned stimulus that is paired with an unconditioned somatosensory stimulus given by maternal care. To establish aversive olfactory learning, an artificial odor can be paired with foot shock during training. TM infused into the bilateral OBs during odor-shock training on postnatal day 11 dose-dependently impaired aversive olfactory learning tested on the next day without affecting memory retention 1 hour after the training. Behavioral pharmacology shows that TM-induced ER stress causes the selective impairment of the long-term memory process of aversive olfactory learning. Electrophysiological experiments using OB slices show that TM administration has an inhibitory effect on the late phase of long-term potentiation induced at the mitral-to-granule cell synapses without affecting the early phase of long-term potentiation. These results are consistent each other to suggest that ER stress impaired aversive olfactory learning by inhibiting synaptic plasticity in the OB.

(COI: No)

P3-163

Two mechanisms underlying maintenance of long-term potentiation at synapses in the mouse accessory olfactory bulb

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Microcircuits in the accessory olfactory bulb (AOB) include the prominent reciprocal dendrodendritic synapse between mitral cells, a single class of projection neurons, and granule cell interneurons. Long-term potentiation (LTP) at the AOB synapse is expected to underlie the pheromonal memory that avoids pregnancy block in mice. We have previously shown that the late-phase LTP at the AOB synapse depends on new protein synthesis. Here we examined whether the late-phase LTP also requires actin polymerization, which is suggested to play a crucial role for LTP maintenance in the hippocampus and the neocortex. Using AOB slice preparations, we measured field potentials (fEPSP) derived from granule cells to examine the effects of an actin polymerization inhibitor or inducer on the late-phase LTP. Tetanic stimulation, consisting of a 100 Hz, 100 pulse train applied four times at 3 min intervals, induced LTP lasting for 180 min. Under bath application of an inhibitor of actin polymerization, cytochalasin D (1 μM), the tetanic stimulation failed to induce the late-phase LTP. The late-phase LTP was induced when subthreshold stimulation (a 100 Hz, 100 pulse train applied twice at 3 min intervals) that only produced short-term potentiation was paired with an inducer of actin polymerization, jasplakinolide (0.2 μM). The results support the hypothesis that both new protein synthesis and actin polymerization underlie LTP maintenance at the AOB synapse.

(COI: No)

P3-164

Modulation of reciprocal synaptic transmission between mitral cells and granule cells in the mouse accessory olfactory bulb through vasopressin V_1 receptors

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Central vasopressin facilitates social recognition and modulates numerous complex social behaviors in mammals. Recent analysis of transgenic rats engineered with an enhanced green fluorescent protein reporter for vasopressin synthesis identified new population of vasopressin neurons in the accessory olfactory bulb (AOB). The AOB has been demonstrated to be a critical site for mating-induced mate recognition (olfactory memory) in female mice. The effect of vasopressin, however, on the synaptic transmission between dendrites in the AOB of female mice is largely unknown.

To address this issue, evoked synaptic currents were measured from mitral cells in slice preparations prepared from 23- to 36-day-old Balb/c mice. To evoke dendrodendritic inhibition, a depolarizing voltage step from -70 mV was applied to a mitral cell under the whole-cell configuration. We have demonstrated that vasopressin significantly reduced the IPSCs in Mg^{2+}-free solution.

In the present study, to determine the contribution of different vosopressin receptor subtypes (V_{1a} and V_{1b}) to reduce the IPSCs, effects of antagonists for V_1Rs on the IPSCs were tested. The suppressive effect of vassopressin on the IPSCs was diminished by an antagonist for V_{1a} receptors, Manning compound, while an antagonist for V_{1b} receptors, SSR149415 unaffected the effects of it. The present results suggest that vasopressin modulates reciprocal transmission between mitral cells and granule cells through vasopressin V_{1a} receptors.

(COI: No)

P3-165

The blockade of $GABA_A$ receptors in the bed nucleus of the stria terminalis further suppresses the intake of conditioned aversive taste solution

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The bed nucleus of the stria terminalis (BNST) receives projections from the taste-related brain regions. However, the involvement of the BNST in conditioned taste aversion has still been unclear. The BNST contains dense GABAergic interneurons. Therefore, we tested the effects of microinjections of $GABA_A$ receptors antagonist bicuculline into the BNST on the intake of conditioned aversive taste solution on the retrieval of CTA. The rats implanted with guide cannulae into the BNST were trained to drink water during a 20-min session. On the conditioning day, all rats received a pairing of a 5 mM saccharin solution with an i.p. injection of 0.15 M lithium chloride. On the retrieval tests (Test 1-3), the rats were presented with the saccharin CS for 20 min. On Test 3, the rats were divided into two groups. One group was microinjected with saline, the other with bicuculline (100 ng/0.25 μl), 30 min before the CS presentation. All rats acquired robust aversion to the CS, because their CS intakes on Test 1 and 2 were significantly lower than those on the conditioning. On Test 3, the bicuculline-injected rats showed significantly lower intake of the saccharin CS than the saline-injected rats. These results indicate that the blockade of $GABA_A$ receptors in the BNST enhance the suppression of the consumption of the CS solution on the retrieval of CTA. Therefore, it is suggested that the GABAergic transmission in the BNST are involved in some taste memory process. This work was supported by JSPS KAKENHI (24500973).

(COI: No)

P3-166

Behavioral and neural mechanisms to ingest vitamin C in vitamin C-deficient rats

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The behavioral and neural mechanisms to ingest sufficient vitamin C (VC) has not been clear. In the present study, we performed the behavioral and the electrophysiological experiments to investigate the mechanisms to ingest sufficient VC. As experimental animals, ODS/Shi Jcl-od/od (od) rats, which cannot synthesize VC in their own metabolism, were used. In the behavioral experiment, the preferences ratio for various concentrations of VC solutions were measured before and after deprivation of VC solution in od rats by using two-bottle preference test. In the electrophysiological experiment, we compared the integrated chorda tympani (CT) nerve response in the VC deficient rats with that in the non-deficient rats. The results were as follows: In the behavioral experiment, the od rats avoided the high concentration of VC solutions on the sufficient VC situation, but this aversive behavior was reduced by the deficiency of VC. On the other hand, preference ratios for low concentration of VC were not changed by the deficiency of VC. In the electrophysiological experiment, the magnitudes of whole CT nerve responses to VC, HCl, NaCl and quinine hydrochloride in od rats with sufficient VC were significantly smaller than those in rats with deficiency of VC. On the other hand, those responses to sucrose and monopotassium glutamate in the VC deficient od rats were as same as those in the normal od rats. These data suggest that rats with sufficient VC can avoid the high concentration of VC solutions by receipting VC as a tastant, but this avoidance is reduced by the deficiency of VC.

(COI: No)

P3-167

Neural pathway contributing to control mechanism of sucrose preference by body weight in mice

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Mice consumed high-caloric sweetener sucrose (Suc) rather than non-caloric sodium saccharin (Sac), when their body weight (BW) reduced to 75% of the original BW by chronic food-restriction. However, Suc consumption abruptly dropped to Sac consumption levels (Saltatory Suppression of Suc Preference, SSSP) immediately after the regain their original BW in 50% mice tested. The SSSP tended to be suppressed by lesions of somatosensory area for the limbs (S1FL/HL). In the present experiment, we examined the expression of a neuronal activity marker c-Fos in S1FL/HL and amygdala to clarify the relationship between those brain regions. The number of c-Fos positive cells in S1FL/HL of pre-SSSP group was significantly lower than that of post-SSSP group. In contrast, the number of c-Fos positive cells in amygdala of pre-SSSP group was higher than that of post-SSSP group. These results suggest that the sensory activation of S1FL/HL encoding increment of BW decreases amygdala function, resulting in suppression of Suc preference. We attempt to examine neural pathway from S1FL/HL to amygdala using anterograde and retrograde tracing.

(COI: No)

P3-168

Glucagon like peptide-1 (GLP-1) underlies sweet taste transmission

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Recent studies demonstrated that taste bud cells express several gut peptides, such as GLP-1 (glucagon like peptide-1), NPY (neuropeptide Y), and glucagon, and secrete these peptides in response to various taste stimuli. Interestingly, the secretion patterns of peptides are correlated with taste qualities, suggesting the possibility that these gut peptides would contribute to taste quality coding. In this study, we report the expression of GLP-1 in some taste cells which possess sweet taste receptor subunit T1R3, and GLP-1 receptor is expressed in gustatory nerve neurons in wild type mice. Mice genetically lacking of GLP-1 receptor showed reduced sweet taste responses in chorda tympani (CT) nerve recordings. GLP-1 is secreted from a subset of sweet responsive cells by sweet taste stimulation in a concentration dependent manner. Furthermore, i.v. injection of GLP-1 produced transient increase of neural activities in a subset of sweet specific single nerve fibers without affecting those of other taste fibers. This activation by injected GLP-1 was observed also in CT nerve of ATP receptor knockout (P2X2/3 KO) mice. All these findings suggest that GLP-1 may be involved in normal sweet taste signal transmission in mice.

(COI: No)

P3-169

Paclitaxel alters sweet-preference in rats

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The alterations in the sense of taste (Dysgeusia) are often overlooked side effects in cancer patients undergoing chemotherapy. Dysgeusia affects the daily quality of life (QOL) of these patients and causes malnutrition in a majority of the patients. However, the research is lacking. The aim of the present experiments was to investigate the effects of paclitaxel (Taxol) on voluntary sucrose-solution intake using a two bottle choice. For this purpose, rats were presented with both a sucrose solution (0.3 M) and water and the consumption of each was measured daily. Usually rats prefer sucrose solution. Intraperitoneal administration of paclitaxel caused a decrease in sucrose-solution intake within a few days after being administered. However, the preference for sucrose solution returned in several days after we stopped. Outcomes indicate paclitaxel attenuates sweet-taste preference in two bottle choice (sucrose vs. water). Since molecular weight of paclitaxel is approximately 850, it cannot pass through the blood brain barrier. These results suggest that paclitaxel affects peripheral taste system or circumventricular organs involved in the sweet taste transduction.

(COI: No)

P3-170

Effects of estrogen on Mu and Kappa opioid inhibition of TMJ-responsive neurons in superficial laminae at the spinomedullary junction in ovariectomized female rats

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Chronic painful temporomandibular joint disorders (TMD) occur more often in women than men and are difficult to manage. There is evidence for significant sex difference in the potency of opioid analgesics in human. The influence of analgesic agents on neurons activated by stimulation of temporomandibular joint (TMJ) region is not well defined. The spinomedullary junction (trigeminal subnucleus caudalis (Vc/C1-2) is major site of termination for TMJ sensory afferents. To determine whether estrogen status influences opioid-induced modulation of TMJ-responsive neurons, mu or kappa opioid agonists (morphine or U50488) was given to ovariectomized (OvX) rats treated for 2days with low-dose (LE2) or high-dose (HE2) estrogen. Under isoflurane anesthesia TMJ units were activated by ATP (1 mM, 20 μl) injected into the joint space before and during cumulative doses of morphine or U50, 488H (0.03- 3mg/kg, iv) given at 20 min intervals. Morphine inhibited evoked activity in units from LE2 rats in a dose-related and naloxone-reversible manner, whereas from HE2 rats were not inhibited. By contrast U50, 488 inhibited evoked activity in units from HE2 rats in a dose-related and nBNI-reversible manner, whereas from LE2 rats were not inhibited. These data indicated that estrogen status differentially affected mu or kappa opioid modulation of TMJ unit activity.

(COI: No)

P3-171

Angiotensin II modulates sweet taste sensitivity via endocannabinoid receptor

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Angiotensin II (AngII) suppresses the chorda tympani (CT) nerve responses to NaCl and enhances those to sweeteners via AngII typeI receptor (AT1) expressed in taste cells. The effect of AngII on sweet taste responses but not on salt taste responses is not observed in cannabinoid receptor (CB1) knock out mice. It is also reported that CB1 is trans-activated by AT1, which induces generation of 2-arachidonoyl glycerol (2-AG), a major endocannabinoid. We, therefore, investigated whether the sweet enhancing effect of AngII is mediated by both CB1 and AT1 using their pharmacological blockers. Intraperitoneal injection of CB1 blocker AM251 specifically inhibited sweet enhancing effect of AngII on the CT nerve responses. Interestingly, we found that repeated taste stimulation with sweeteners but not with other tastants gradually increased individual responses. AngII enhanced the effects of repeated taste stimulation with sweeteners. AM251 or AT1 blocker CV11974 inhibited the AngII's effects. Taken together, we presumed that 2-AG would be produced via sweet taste receptor and/or AT1 activation, leading to enhancement of sweet taste responses via CB1.

(COI: No)

P3-172

The functional analysis of Mash1 in mouse taste bud cell differentiation using Cre-loxP system

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The gustatory cells in taste buds have been identified as paraneuron, they possess characteristics of both neuronal and epithelial cells. Like neurons, they form synapses, store and release transmitters, and are capable of generating an action potential. Like epithelial cells, taste cells have a limited life span and are regularly replaced throughout life. However, little is known about the molecular mechanisms that regulate taste cell genesis and differentiation. In the present study, to begin to understand the mechanisms that regulate taste bud cell differentiation, we have investigated the role of Mash1 in regulating taste bud cell differentiation in Mash1 conditional knockout mice (CKO) using Cre-loxP system. We found that amino acid decarboxylase-immunoreactive (AADC-IR) cells and carbonic anhydrase 4-immunoreactive (CA4-IR) cells were significantly reduced in the circumvallate papilla epithelia of Mash1 CKO mice. In Mash1 CKO/GAD67-GFP mice, GFP-positive (GAD67 expression type III cell) cells were also reduced in the taste papilla epithelia. However gustducin, a marker of type II taste bud cells, was expressed in taste buds in the soft palates of Mash1 CKO mice. These results suggest that Mash1 could play an important role of differentiation of the type III cells in the taste bud.

(COI: No)

P3-173

Leptin's effect on sweet responses is mediated by leptin receptor Ob-Rb and metabolic sensor K_{ATP} channel

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Leptin selectively suppresses neural and behavioral responses to sweet tastants. However, the molecular and cellular basis for the specific link between leptin and sweet taste still has not been elucidated. Here, we report that sweet suppressive effect of leptin is mediated by functional leptin receptor (Ob-Rb) and ATP gated K^+ (K_{ATP}) channel expressed in sweet sensitive taste cells. Ob-Rb was much more abundantly expressed in taste cells expressing T1R3 (a sweet receptor component) than in those expressing GLAST (a Type I cell marker) or GAD67 (a Type III cell marker). Administration of leptin suppressed sweet but not bitter and sour responses of identified taste cells. This effect was inhibited by leptin antagonist and was not observed in leptin receptor deficient *db/db* mice and also in diet induced obese (DIO) mice. K_{ATP} channel subunit SUR1 was well coexpressed with Ob-Rb in T1R3 expressing taste cells and sweet suppressive effect of leptin was inhibited by addition of K_{ATP} channel blocker glibenclamide in dose dependent manner. In addition, K_{ATP} channel activator diazoxide mimicked the sweet suppressive effect of leptin. These results indicate that leptin suppresses taste responses of sweet sensitive taste cells via activation of Ob-Rb and K_{ATP} channel.

(COI: No)

P3-174

Glucuronosyl group of gymnemic acids mainly interacts with the transmembrane domain of human T1R3 in sweet-suppressing effect

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Gymnemic acids (GAs) are triterpene glycosides isolated from the plant *Gymnema sylvestre*. GAs are known to selectively suppress taste responses to various sweet substances in humans, but not in mice. This effect of GAs is diminished by rinsing the tongue with γ-cyclodextrin (γ-CD). Here we focus on the molecular mechanisms for sweet-suppressing effect of GAs. We examined the interaction between GAs and sweet receptor by utilizing a sweet receptor assay based on changes in intracellular calcium activity in HEK293 cells expressing T1R2 + T1R3. Similar to previous studies in humans and mice, GAs suppressed the $[Ca^{2+}]_i$ responses to sweet compounds in HEK293 cells expressing human but not mouse sweet receptor. This effect of GAs rapidly disappeared after rinsing the cells with γ-CD. Using full length or chimera receptors in human and/or mouse, we determined that the transmembrane domain of hT1R3 was mainly required for the effect of GAs. Glucuronic acid, common structure of GAs, also showed sweet-suppressing effect. In our molecular models, GAs were predicted to dock to a binding pocket within the transmembrane domain of hT1R3.

(COI: No)

P3-175

Morphology of P2X3-immunoreactive nerve endings around laryngeal chemosensory cells in rat

Yamamoto, Yoshio; Takahashi, Natsumi; Nakamuta, Nobuaki (*Iwate Univ., Morioka, Japan*)

Taste bud-like chemosensory cell clusters and solitary chemosensory cells (SCC) are distributed in the laryngeal mucosa. It has been reported that the laryngeal chemosensory cells were immunoreactive to molecules for taste transduction such as α-gustducin and PLC β2. In the present study, morphology of P2X3-immunoreactive nerve endings around laryngeal chemosensory cells was examined using multilabeling immunofluorescence with confocal laser microscopy. In the taste bud-like chemosensory clusters, P2X3-immunoreactive nerve endings were intruded from basal part and ramified in the cluster. Terminal regions of the P2X3-immunoreactive nerve endings were flattened in shape, and attached with spindle cells immunoreactive to α-gustducin or IP3R3, which are markers for type II gustatory cell, and with the slender cells with immunoreactivity for SNAP25 or syntaxin-1, which are markers for type III cell. On the other hand, solitary chemosensory cells were classified into two types, i.e., spindle cells with α-gustducin and IP3R3 immunoreactivities and flask-shaped cells with SNAP25 and syntaxin-1 immunoreactivities. Axon terminals from branched P2X3-immunoreactive nerve fibers were terminated in both types of the solitary chemosensory cells. P2X3-immunoreactive nerve endings were also immunoreactive to vGLUT1 and vGLUT2, but not immunoreactive to CGRP. These results suggest laryngeal chemosensory cells are similar to type II and type III cells in the lingual taste buds, and P2X3-immunoreactive nerve endings have an important role on signal transmission from laryngeal chemosensory cells.

(COI: No)

P3-176

Movement-specific employment of sensory signals as a basis for rapid task switching

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Much of our flexible behavior is dependent on responding efficiently to relevant information, while discarding irrelevant information. In perceptual decision making, a popular hypothesis for utilizing relevant information is that sensory signals are gated, such that only relevant sensory signals reach decision making circuits. Little is known, however, about how neural pathways governing sensory-motor associations can rapidly switch to accomplish such flexibility. Here, we investigated how the outputs of sensory neurons change in a context in which task demand switches rapidly. We addressed this question by electrically microstimulating middle temporal (MT) neurons selective for both motion direction and binocular disparity in monkeys switching between direction and depth discrimination tasks. We frequently found that the observed psychophysical bias precipitated by delivering microstimulation to neurons whose preferred direction and depth were related to opposite choices in the two tasks (incongruent sites) was substantially shifted toward a specific movement. Furthermore, the degree of movement-specific employment was correlated with how well the monkey ignored the irrelevant information. Our findings suggest that the outputs of sensory signals are movement-specific, and that irrelevant sensory-motor pathways are filtered out depending on task demand, to accomplish rapid attentional switching.

(COI: No)

P3-177

Experience-Dependent Clustering of Sensory Synaptic Inputs in the somatosensory cortex

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Experience-dependent clustered synaptic plasticity is thought to underlie the formation of highly structured synaptic input patterns. However, direct experimental evidence for this hypothesis is lacking. To directly test the effect of sensory experience on synaptic input patterns, we analyzed individual synaptic inputs by using in vivo two-photon calcium imaging and whole-cell recordings from layer 2/3 neurons in the mouse barrel cortex. We found that both sensory-evoked synaptic inputs in local dendrites were sparse but locally clustered, and the majority of sensory inputs were localized to a small subset of spines. Importantly, whisker deprivation during the critical period significantly enhanced co-activation of distant spines. These results suggest that sensory information is represented by highly heterogeneous and structured synaptic inputs that are shaped by sensory experience during the critical period.

(COI: No)

P3-178

Effects of forelimb stimulation on the propagating excitatory wave evoked by hindlimb stimulation in the rat sensorimotor cortex recorded with optical recording system

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We have developed an optical recording system using a voltage-sensitive dye. Using this system, we have reported that the neural excitation induced by a somatic stimulation initiates from the somatotopically corresponding site and spreads outward in the sensorimotor cortex like the wave produced when a stone is dropped into still water. In this study, we examined the influence of forelimb stimulation on the hindlimb stimulation-induced response. The sensorimotor cortex was exposed and stained with a voltage sensitive dye (RH-414). An electrical stimulation (1 mA, 0.5 ms) was given first to the hindlimb then to the forelimb. The interstimulus interval (ISI) was 0, 5, 10, 15 or 20 ms. We compared the amplitude, full width at half maximum (FWHM) and slope of the rising phase of the hindlimb-response signal between with and without forelimb stimulation, at the collision position of the two propagation waves. The collision position was estimated from the isochrone maps based on the latency difference of each pixel. The FWHM was significantly shorter for all ISIs. The slope of rising phase was significantly steeper but only for the ISI of 0 ms. No significant difference was found in the amplitude for any ISIs. In addition, the latency of the hindlimb-response at its initiation site was shorter than the control, for the ISIs of 0 and 5 ms. Thus, the effects of subsequent stimulation were not of simple summation but of complicated phenomena depending on the ISI.

(COI: No)

P3-179

Discrimination of optogenetic whisker pattern in channelrhodopsin-2 transgenic rat

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The rodent whisker-barrel cortical system has been a model to study somatosensory representation in the cortex. Optogenetics would facilitate this with highspatiotemporal resolutions. Recently, we have identified the expression of channelrhodopusin-2(ChR2) in the mechanoreceptive neurons in the trigeminal ganglion in one of Thy1.2-ChR2-Venus transgenic rat lines, W-TChR2V4. Each whisker follicles were thus innervated by ChR2-positive nerve endings. Here, we studied if this rat can discriminate the irradiation patterns on their whiskers. A W-TChR2V4 rat was fixed its in head in awake and irradiated blue LED light on each whisker as a signal of operant conditioning of either Go or No-go task. The Go task was designed so as the rat is allowed to get a reward, when it licked the nozzle within 5s after whisker irradiation. The No-go task was designed so as, the rat have to withhold licking least 5s to get a reward. After training with blue light the W-TChR2V4 rat performed Go task, with success rate of over 80%. However, the success rate was less than 50% with red LED light or the wild type rat that did not express ChR2. When switched from Go to No-go task, once decreased success rate increased again with the repeat of training sessions. One of two points on the same side was irradiated as a Go signal and another as a No-go signal. It learned to discriminate these patterns successively with sessions and even with days. It is suggested that the optogenetic approach would facilitate to study how the spatiotempolal pattern of the mechanoreception would be interpreted in the cortex.

(COI: No)

P3-180

Somatosensory Cortical Responses after Crossing Nerve Transfer in Mice

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To understand the therapeutic effects of crossing nerve transfer for brachial plexus injuries in human patients, we investigated cortical changes after crossing nerve transfer of brachial plexus using flavoprotein fluorescence imaging in mice. The distal cut ends of the left median and ulnar nerves were connected to the central cut ends of the right median and ulnar nerves with a sciatic nerve graft at 8 weeks old. After eight weeks, responses in the primary somatosensory cortex (S1) elicited by vibratory stimulation applied to the left forepaw were visualized. In control mice, direct responses (DRs) mediated via thalamic input was observed in the contralateral S1. Weak indirect responses (IRs) were also observed in the ipsilateral S1. In nerve crossing mice, DRs were observed in the ipsilateral S1. At the same time, clear IRs, which were not observed in control mice, were found in the contralateral S1. In our previous study, it was expected that DRs were initiated by thalamic inputs to layer 4, while IRs were secondarily initiated by callosal inputs from the ipsilateral S1 to the layer 2/3. We next perform the experiment using macroconfocal microscope to analyze the latencices of activity in each layer. After analyzing every layer about DRs of the control mice and IRs of the experiment mice, layer 4 was dominant with control mice, and layer 2/3 was dominant with the nerve crossing mice.

(COI: No)

P3-181

Opt-fMRI study of whisker-barrel cortical responses using channelrhodopsin-2 expressing rat

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The rodent whisker-barrel cortical system has been a model to reveal somatosensory representation in the brain. Optogenetics would facilitate this with high spatiotemporal resolutions. Recently, we have identified the expression of ChR2 in the mechanoreceptive neurons in the trigeminal ganglion (TG) in one of thy1.2-channelrhodopsin 2 (ChR2)-Venus transgenic rat lines, W-TChR2V4 (Honjoh et al., 2014). Each whisker follicles were also richly innervated by the ChR2-positive nerve endings. The whiskers of a ChR2-expressing rat were all trimmed and 16 follicles were attached with plastic optic fibers in array of 4x4. Each endings of optic fiber was connected to blue LED, which turned on and off independently by a pulse generated by a computer. Therefore, the whiskers could be stimulated with various spatiotemporal patterns (optogenetic tactile pattern, OTP). The functional magnetic resonance imaging (fMRI) responses of barrel cortex were investigated using OTP under 7T-MRI system. The whisker irradiation induced a change of blood oxygenation level-dependent (BOLD) responses in the barrel field of contralateral somatosensory cortex in a manner dependent on time. The response to a single whisker irradiation was more regional than the simultaneous 16-whiskers irradiation. It is suggested that the OTP was accompanied with specific spatiotemporal changes of BOLD response. Our OTP, in combination with fMRI, would facilitate to study how the spatiotemporal pattern of the whisker mechanoreception would be represented in the cortex.

(COI: No)

P3-182

Peripheral nerve injury changes neuronal firing patterns in the somatosensory thalamus of unanesthetized mice

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Peripheral nerve injury induces massive remodeling in the central nervous system. Recently, we have demonstrated that transection of the peripheral sensory nerve increases excitatory afferent fiber innervations onto a relay neuron in the somatosensory thalamus (VPm neuron) [Takeuchi Y. *et al*. (2012) J Neurosci 32: 6917]. On the other hand, the transection also increases tonic inhibitory membrane conductance in a VPm neuron (Nagumo Y. *et al*., unpublished data). However, it is currently unknown about the net effects of such changes on VPm neuronal firing in a physiological condition. To address this issue, we here developed extracellular recordings of VPm neuronal firing from unanesthetized young adult mice using a U-frame head holder [Chiken S. *et al*. (2008) J Neurosci 28: 13967]. One week after the transection, spontaneous tonic (non-burst) spike frequency significantly decreased (6.5 ± 5.8 Hz vs. 3.8 ± 4.3 Hz, mean ± s.d., 30 and 41 neurons for control and transection groups, respectively; $^{*}P < 0.05$, Mann-Whitney *U*-test) whereas burst spike frequency did not (2.6 ± 3.6 Hz vs. 1.3 ± 1.3 Hz; $P = 0.34$). Consequently, the proportion of burst spikes to total spikes significantly increased (0.22 ± 0.16 vs. 0.31 ± 0.18; $^{**}P < 0.01$). These results suggest that VPm neuronal firing is globally inhibited by increased tonic inhibitory conductance and shifted from tonic to burst firing mode after the transection.

(COI: No)

P3-183

Parvalbumin-expressing lamina II interneurons are an origin of presynaptic inhibitory inputs on to central terminals of myelinated low-threshold mechano-receptors in the rodent spinal cord

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Axo-axonic synapses have been described on the central terminals of tactile afferents and certain classes of nociceptive fibers, however the origin of these inputs have yet to be identified. A recent report has shown that a significant proportion of inhibitory inputs on to the central terminals of myelinated afferents terminating in lamina II inner (IIi) of the mouse spinal dorsal horn express parvalbumin (PV) and that these inhibitory boutons are likely to be derived from PV-expressing cells in laminae IIi and III. In this study, we aim to confirm the origin of these PV-expressing axo-axonic inputs. We have used in vitro whole-cell patch-clamping techniques to record from and label PV-expressing cells in lamina IIi-III. We have observed that most of the axon terminals from all of the PV-expressing cells recovered form inhibitory presynaptic inputs on to the central terminals of myelinated afferents. Our findings confirm that PV-expressing cells in laminae IIi and III are a source of inhibitory presynaptic inputs on to the central terminals of myelinated afferents. These cells might play an important role in the development of tactile allodynia.

(COI: No)

P3-184

Repeated forced swim stress enhances CFA-evoked mechanical hypersensitivity and affects the expressions of pCREB and delta-FosB and the acetylation of histone H3 in the insular cortex

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Exposure to stressors causes substantial effects on the perception and response to pain. In several animal models, chronic stress produces lasting hyperalgesia. The insular (IC) and anterior cingulate cortices (ACC) are the regions exhibiting most reliable pain-related activity. And the IC and ACC play an important role in pain modulation via descending pain modulatory system. In the present study we examined the expressions of pCREB and delta-FosB and the acetylation of histone H3 in the IC and ACC after forced swim stress and CFA injection to clarify changes in the cerebral cortices that affect the activity of descending pain modulatory system in the rats with stress-induced hyperalgesia. Forced swim stress (day 1, 10min; days 2-3, 20min) or CFA injection into the hindpaw induced a significant increase in the expressions of pCREB and delta-FosB in the IC. However the forced swim stress prior to CFA injection showed significant enhancement of CFA-evoked mechanical hypersensitivity and attenuation of the increase in the expressions of pCREB and delta-FosB in in the IC. Cells of the IC also displayed evidence of chromatin remodeling. The forced swim stress prior to CFA injection attenuated the CFA-evoked increase in acetylation of histone H3 in the IC. These findings suggest neuroplastic and epigenetic changes in the IC after forced swim stress, which may be involved in the enhancement of CFA-induced mechanical hypersensitivity through dysfunction of descending pain modulatory system.

(COI: No)

P3-185

Opposing role of NMDA receptor GluN2B and GluN2D in somatosensory development and maturation

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NMDA receptors are essential for activity-dependent synapse refinement, and thus required for correct somatosensory map formation. However, distinct role of each NMDA receptor subunit remains largely unknown. Here we investigated functional roles of GluN2B (GluR ε 2 or NR2B) and GluN2D (GluR ε 4 or NR2D) in development of whisker-related patterning at trigeminal relay stations. Compared to control littermates, both the appearance of whisker-related patterning and the termination of the critical period plasticity were delayed by nearly a day in each of the trigeminal relay station of GluN2B+/- mice, while advanced by nearly a day in GluN2D-/- mice. Importantly, these temporal shifts were not accompanied by changes in the magnitude of lesion-induced critical period plasticity. Thus, GluN2B and GluN2D play counteractive roles in temporal development and maturation of somatosensory maps. Further analysis revealed that GluN2B was predominantly expressed in non-GABAergic neurons, while GluN2D was selective to GABAergic neurons in each of the trigeminal relay station. Taken together, our findings suggest that GluN2B expressed in ascending projection pathway and GluN2D expressed in inhibitory circuit accelerates and decelerates somatosensory map development, respectively.

(COI: No)

P3-186

The immunohistochemical characterization of the new heat-sensitive primary sensory neuron in mouse dorsal root ganglia

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Transient receptor potential vanilloid 1 and 2 (TRPV1 and V2) has been proposed as the heat sensors in nociceptors. However, several reports did not support major contribution of TRPV2 in heat nociception. We previously reported a new class of the heat-sensitive neurons in mouse dorsal root ganglia (DRG) using immunohistochemical detection of heat-induced phosphorylation of extracellular signal-regulating kinase (pERK). To characterize these neurons, here, we examined them by double-staining immunohistochemistry with antibodies against the marker molecules for the nociceptive neurons. In wild-type mice, ~20% of the neurons were pERK-positive after heat stimuli. The number of the heat-induced pERK-positive neurons was reduced but significantly observed in TRPV1-deficient mice. The most of the pERK-positive DRG neurons were TRPV2-negative, but positive to other molecular markers for the nociceptive neurons, e.g. CGRP (calcitonin-gene related peptide). The results suggest that the new heat-sensitive neurons share the features of nociceptive neurons, but their heat-sensitivities are independent of TRPV1/V2.

(COI: No)

P3-187

Primary nociceptive modulation via pigmentation-dependent dopaminergic signaling in skin

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In human and mice, pigmentation level is highly correlated to heat pain sensitivity in the skin. Tyrosinase does not only control melanin production, but also produces L-dopa that is converted into dopamine. To examine whether the peripheral dopamine mediates pain sensitivity, we investigated effects of local dopamine injection on nociceptive responses and nociceptive receptor expressions on primary sensory neurons in black C57BL/6 (B6) and albino tyrosinase-mutated B6 (B6(Cg)-*Tyr*$^{c-2J}$) mice. In the hind paw and whisker pad, B6 showed significantly lower mechanical and higher thermal sensitivities than albino B6. Subcutaneous injection of dopamine produced sustained hyposensitivity to mechanical stimulation and hypersensitivity to thermal stimulation. The same sustained changes in mechanical and thermal sensitivity were seen after injection of L-dopa or D_1 agonist SKF38393, but not injection of catecholamines or other dopamine receptor subtype agonists. The tyrosinase inhibitor kojic acid and the D_1 antagonist SCH23390 showed opposite effects on mechanical and thermal sensitivities. Injection of dopamine and SKF38393 into the whisker pad also downregulated mRNA expression of the mechano-sensitive receptor Piezo2, and upregulated mRNA expression of the heat-sensitive receptor TRPV1, in the associated trigeminal ganglia. These results suggest that tyrosinase-dependent dopamine production mediates expression levels of nociceptive receptors in sensory neurons via D_1 activation.

(COI: No)

P3-188

Three dimensional reconstruction of trigeminal ganglion cell processes labeled by intracellular injection: Emphasis on club-like endings

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Primary somatosensory neurons are pseudo-unipolar cells. Both the peripheral and central endings of the neurons, as well as their firing characteristics, were identified and characterized, using intracellular labeling and recording in the rat trigeminal ganglion in vivo. Eleven of 35 labeled neurons terminated as club-like endings (Clubs) in the whisker follicles. All the neurons responded to deflection of their corresponding whisker. Eight of the Club neurons never ramified in the peripheral branch. They indicated significantly shorter duration at base time of action potential (1.7 ± 0.6ms) in contrast to the other endings (n=27; 2.5 ± 1.0ms). Analyses of serial semi-thin sections showed 52 Clubs to be arranged side by side along a cylindrically-shaped narrow belt zone around the follicle at connecting level of the ringwulst (Rw). Our findings indicate that approximately 50 neurons innervate a single small zone of the Rw. Rw is a sausage like protrusion into the ring sinus. Clubs were connected with collagen fibers extending from the Rw. We propose that the Clubs are sensitive to momentum changes of the Rw during whisker protraction or retraction.

(COI: No)

P3-189

Three-dimensional distribution of lamellar corpuscles in a human toe

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We investigated three-dimensional distribution of lamellar corpuscles and their afferents in a human infant toe. A distal part of a toe excised from a polydactyl patient (1year-old; informed consented by parents and obtained the permission of Hospital Ethics Committee of Kyoto Prefect. Univ. Med.). The toe was fixed, decalcified and cut into 80 μm-thick serial sections and immunohistochemically stained using primary antibodies against protein gene product 9.5 and myelin basic protein. Pacinian corpuscles (PCs) showed relatively simple and straight axon terminals surrounded by lamellae and Golgi-Mazzoni corpuscles (GMCs) displayed ramified and tangled ones. All PCs and GMCs were mapped on a 3D image. Totally 51 corpuscles (47 PCs and 4 GMCs) existed. Diameters (transverse / longitudinal) of these PCs and GMCs were 0.1-0.7 / 0.2-1.6 and 0.1-0.3 / 0.2-0.5 mm, respectively. 42 PCs were distributed in ventral, the rest 5 PCs were dorsal and all the 4 GMCs were in the lateral side of the toe. All corpuscles were located in the subcutis or deeper but not in the dermis. 33 PCs existed close to the distal phalange but little was beneath it. 14 PCs and 4 GMCs existed in between the tendons and the middle phalange. 10 corpuscles were solitarily scattered, but 41 distributed in 12 groups as 2-7 corpuscles. These observations may contribute to consider three-dimensional architecture of sensing mechanisms in the human finger.

(COI: No)

P3-190

In vivo analysis of visceral sensory inputs to the sacral spinal cord

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Sacral spinal dorsal horn receives synaptic inputs not only from somatic afferent but also from pelvic afferent fibers, and has important roles in integrating the sensory information and controlling functions of pelvic organs including the lower urinary tract functions. Sensory information including pain from the pelvic organs is known to be conveyed to the sacral dorsal horn by small myelinated A δ - and unmyelinated C-fibers. However, little is known about how the sacral dorsal horn receives pelvic sensory information *in vivo*. In the present study, we examined sacral spinal sensory responses from the lower urinary tracts by using *in vivo* patch-clamp and extracellular recording techniques. Sacral dorsal horn (SDH) neurons *in vivo* elicited action potentials in response to bladder filling and voiding. During the micturition cycle, the SDH neurons were classified into two types based on their responsiveness: neurons showing bursts of action potentials that correlated to the rise in intravesical pressure during voiding, and neurons which elicited firings at the peak pressure. Both groups of SDH neurons received inputs from slow conducting afferent fibers and the latter groups of neurons elicited firing by electrical urethra stimulation. These results suggest that sensory information from the bladder and urethra was separately conveyed to the sacral dorsal horn, and the precise pattern of spike timing in each group of SDH neurons may be needed to implement appropriate micturition.

(COI: No)

P3-191

Ultrastructural analysis of itch-related neural network in the spinal cord

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Gastrin-releasing peptide (GRP) has been reported as an itch mediator in the somatosensory system. We demonstrated that GRP was expressed in the small-sized primary afferent neurons, and central axons terminated the superficial layers of spinal dorsal horn and spinal trigeminal nucleus caudalis in rats. Furthermore, ultrastructure of GRP containing axon terminals in the spinal dorsal horn was analyzed by the following electron immunecytochemistry: high-voltage electron microscopy showed GRP containing axon terminals of a series of the varicosities; transmission electron microscopy displayed GRP expressing presynaptic terminals containing excitatory neurotransmitters; 3D-scanning electron microscopy showed GRP containing varicosity surrounded by tens of postsynapses. These results suggested that itch transmission is elaborately controlled by many synapses in the spinal dorsal horn.

(COI: No)

P3-192

Effects of neurotoxic destruction of noradrenergic fibers of the central nucleus of amygdala on rat inflammatory orofacial pain

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The amygdala is a kernel site for chronic pain-induced emotional complications. It receives non-thalamocortical direct inputs through spino- (parabrachio-) amygdaloid pathway, which exhibits robust synaptic potentiation in a variety of chronic pain models. Although it is well documented that exogenous noradrenaline potently modulates neuronal activity and synaptic transmission in the amygdala, the role of endogenous noradrenaline in the amygdala in the various symptoms observed during pain chronification has been only poorly addressed. We injected saporin conjugated with anti-dopamine-beta-hydroxylase (DBH) into the central nucleus of amygdala (CeA) and compared the various behavioral consequences in the inflammatory pain model. To allow evaluation of voluntary choice of floors of different temperatures (thermal preference test, TP) by rats with inflammatory pain, we injected formalin into orofacial regions, which resulted in manifest nocifensive behaviors lasting for < 60 min, and evaluated changes in paw withdrawal threshold (PWT) and TP index for >24 h after formalin injection. Saporin injection into the CeC resulted in elimination of DBH-immunopositive fibers in the CeC and also a partial reduction of DBH-immunpositive neurons in the locus coeruleus. On the basis of distinct effects of saporin treatment on PWT and TP, noradrenaline system might play differential roles in the regulation of nocifensive spinal reflex and voluntary choice of sub-aversive environments.

(COI: No)

P3-193

Functional characterization of pruriceptive dorsal root ganglion neurons in rats

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Itch sensation is different from painful one. However, it has been known that subsets of nociceptive sensory neurons mediate itch sensation. The fundamental question is how the peripheral neuronal activities specificially mediate itch sensation. Here, we used an original method for *in vivo* patch-clamp recording to allow integrated analysis of the diverse properties of dorsal root ganglion (DRG) neurons in rats and investigated the characteristics of chloroqunie (CQ; one of itch-induced chemical agents)-sensitive DRG neurons. Small- and medium-sized DRG neurons that innervate the skin were screened according to axonal conduction velocity (C-type and A δ-type), action potential duration, current expression profiles (Ih, IA, and T-Ca) and could be classified into 5 classes (Class I-V). Intradermal injection of CQ to the receptive field evoked discharges in some DRG neurons that belonged to Class I characterized by a long action potential and small Ih or to Class II characterized by a shorter action potential and IA. The Class I neurons were high-threshold mechanosensitive and also responded to heat (42-55℃) or warm (32-40℃) stimulation to the receptive field, whereas the Class II ones were moderate-threshold mechanosensitive and heat sensitive. All of the somata responded to application of capsaicin. Comparing with the characteristics of CQ-insensitive and nociceptive DRG neurons, we will discuss encoding mechanisms of itch sensation evoked by CQ.

(COI: No)

P3-194

Neural mechanisms of nociception in an animal model of fibromyalgia

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Chronic widespread pain is a serious medical problem, yet the neural mechanisms remain to be elucidated. Using a reserpine-induced animal model for fibromyalgia, this study was undertaken to examine: 1) the expression of pain-related ion channels in the dorsal root ganglion (DRG); 2) activities of peripheral nociceptors; and 3) alterations in spinal microglial cells. Acid-sensing ion channel (ASIC)-3 mRNA was significantly upregulated in the DRG, and a selective blockade of this channel was significantly reversed the behavioral mechanical hyperalgesia. Facilitated mechanical responses of mechano-responsive C-fibers both in the skin and muscle were observed. Spinal microglia labeled with Iba1-immunoreactivity was obviously activated, especially in the laminae I-II. The activated microglia and behavioral hyperalgesia were significantly prevented by a minocycline application. These results suggest that the increase in ASIC3 channels in the DRG, facilitated mechanical response of C-nociceptors, and activated spinal microglia may direct to intensify pain in this model. Pain may be further amplified by reserpine-mediated monoamine depletion in the descending pain inhibitory system (Nagakura et al. Pain 2009).

(COI: No)

P3-195

Psychosocial stress by partner-loss enhanced pain behaviors in monogamous animal, prairie voles

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Nociception is modulated by social environmental factors and stressful events. In particular, social stressors may have adverse effects on psychological and nociceptive functioning. Although "stress-induced hyperalgesia" are common clinical symptoms in chronic pain disorders, the mechanisms have been obscure because of a lack of appropriate animal models. The prairie vole is a socially monogamous rodent that exhibits a partner preference after the formation of the pair bonding. Here we analyzed the effect of partner-loss on anxiety behaviors, sensory thresholds, and pain behaviors of the prairie voles. Adult male voles were paired with strange females for 7 days then tested in a 2-h partner preference test at Day 7. Males that displayed a partner preference were divided into paired or partner-loss group. After that, an array of behavioral testing was conducted on males from both groups. Partner-loss males showed much anxiety behaviors in open field test (Day 11), low threshold of mechanical and thermal stimulus in plantar test and von Frey test (Day 12), and much pain behaviors in formalin test (Day 13) as compared to paired males. Spinal dorsal horn and descending inhibitory pathways from the brainstem play crucial roles in nociceptive processing, therefore, we are proceeding to estimate their activity by cFos immunoreactivity and will report the results on the day.

(COI: No)

P3-196

Joint immobilization by cast modulates not only pain threshold but also itch sensation

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Immobilization of joint by cast is commonly used for resting the injured joint. However the reduction of pain threshold for mechanical stimuli is often induced by cast immobilization of the hind limb. The cast treatment also elicits the itch sensation that we have experienced much stress and affect patient's quality of life. In this study using rats, we examined whether the cast treatment modulates the pain threshold to mechanical stimulation and the itch sensation induced by intradermal injection of serotonin. To examine the effects of cast immobilization on pain and itching behaviors in rats, one hind limb was immobilized for 2 weeks with a cast and observation of behavior was conducted after cast removal for 3 weeks. A wire mesh cast was wrapped around the one hind limb to keep the ankle joint almost straight. The pain threshold was measured by using calibrated von Frey filament test before and after cast immobilization. The joint immobilization elicited the reduction of pain threshold which continued almost over 10 day period after a cast removal. The itch sensation was assessed in rats with cast treatment by using the intraplantar injection of serotonin. The serotonin-induced itch sensation as licking and biting behavior was escalated in the rats with cast treatment than that of sham treatment rats. These results suggest that the joint immobilization by the cast not only reduce the pain threshold but also facilitates the itch sensation induced by intradermal injection of serotonin.

(COI: No)

P3-197

Noradrenaline enhances visual detectability via β adrenergic receptor in freely moving rat

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Noradrenaline (NA) is thought to modulate various brain functions such as sensory information processing. Previous electrophysiological studies revealed that iontophoretically-administered NA enhanced the signal-to-noise ratio in the primary visual cortex. It suggests that NA released into the visual cortex improves the visual detectability. However, this point has not been investigated in freely moving rat. In this study, to evaluate the effect of the endogenous NA by adrenergic receptor types, we measured the contrast sensitivity (CS) of Long-Evans rat in two-alternative forced-choice (2AFC) visual detection task combined with staircase method with or without adrenergic blockade. We applied α_2 receptor antagonist, idazoxan (IDA), and β receptor antagonist, propranolol (PRP), intraperitoneally 30 min before the test. We found that IDA increased the CS, whereas PRP decreased one. However, both of these receptors must work coordinately in the natural state, so it was unclear whether endogenous NA increased or decreased the CS. To answer this question, we administered the cocktail of both drugs resulting in the reduction of CS as with β antagonist. Our results demonstrated that endogenously released NA enhanced the contrast detectability, and suggested that the β receptor might work dominantly in freely moving rat.

(COI: No)

P3-198

Effects of neonatal dopamine depletion on itch and pain related responses in the adult rats

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Previous studies have shown that dopamine (DA) system in not only involved in motor control but also modulation of somatosensory information. Rats with DA depletion during adulthood and neonatal period exhibited akinetic motor activity and spontaneous motor hyperactivity, respectively, indicating that behavioral effects of DA depletion depend on the period of lesion development. Although itch and pain have some similarities, they are different sensation, and roles of DA system in development of the systems of their sensations are still unclear. To clarify effects of neonatal DA depletion on response to pruritic and noxious stimuli during adulthood, we analyzed the behavioral response and c-Fos immunoreactivity (Fos-ir) of spinal dorsal neurons, to injection of serotonin (5-HT) or formalin into the hindpaw in adult rats with neonatal 6-hydroxydopamine treatment. Rats with neonatal DA depletion showed significant increases in the numbers of flinch evoked by 5-HT or formalin injections, and decreases in the number and starting time of biting evoked by 5-HT injection. The numbers of Fos-ir spinal neurons evoked by 5-HT or formalin injections were not affected by neonatal DA depletion, while localization of Fos-ir neurons in the spinal cord evoked by 5-HT injection were different from that evoked by formalin injection. These results suggest that a role of DA system in development of somatosensory system depend on the modality, and the spinal neural circuit for pruritic transmission is not always consistent with nociceptive transmission.

(COI: No)

P3-199

Effects of motor cortex stimuli on rostral ventromedial medulla (RVM) cell activity in spared nerve injury (SNI) rats

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Motor cortex stimuli provides anti-nociceptive effects in chronic pain model rats. However the precise mechanisms of anti-nociception remains to be unknown. In the previous study we have shown the possible involvements of rostral ventromedial medulla (RVM)-on and off cells in cortex stimuli-induced pain relief in chronic constriction injury (CCI)rats. In the present study, using spared nerve injury (SNI) rats, another model of chronic pain made by section of common peroneal and tibial nerve but sparing sural nerve, we re-examined the RVM involvement in this motor cortex stimuli-induced anti nociceptive effects. Single unit activity of the RVM cells were recorded with tungsten/stainless steel microelectrodes under pentobarbital anesthesia. Prior to cortical stimuli, the RVM cells were classified into three groups, on-, off-, and neutral cells, based on their responses to nociceptive pinch stimuli applied at the hind paw. Cortical stimulus current intensity was ranged 30-110 μA. We found that spontaneous activity of off cells facilitated whereas on cells inhibited by cortex stimuli in SNI rats for at least 30 minutes after the stimuli. Taken together with previous results of CCI rats, these results suggest RVM is involved in cortex stimuli induced anti-nociceptive effects in chronic pain.

(COI: No)

P3-200

Degenerative histological alteration is not required for the induction of muscular mechanical hyperalgesia after lengthening contraction in rats

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Mechanical hyperalgesia after lengthening contraction (LC) severely restricts physical activities in daily life. The current study aimed to test a hypothesis that degenerative alteration in the exercised muscle is necessary for LC-induced mechanical hyperalgesia. Under isoflurane anesthesia, the tibialis anterior (TA) muscle of rats was loaded repetitive LC of different stretch range of motion (ROM) and angular velocity (VEL). The degree of mechanical hyperalgesia was quantified by measuring the mechanical withdrawal threshold of the exercised muscle before, 3 hours, 1-5 days after LC. Mechanical hyperalgesia appeared after LC at the ROM of 60, 90, and 120° in a ROM-dependent manner while that of 30° did not, and after LC at the VEL of 100, 200, and 400°/s in a VEL-dependent manner while that of 50°/s did not. Degenerative histological change in the TA muscle was observed only in some cases. However, median cross sectional area occupied with degenerated fibers in the TA muscle was 0~0.27 mm2 in all LC protocols used in this study. The area corresponds to 0~0.8% in the TA. These results suggest that mechanical hyperalgesia was induced by LC in a ROM- and VEL-dependent manner, and that massive degenerative change is not required for the induction of mechanical hyperalgesia after LC.

(COI: No)

P3-201

Involvement of Fractalkine (FKN) in ectopic orofacial pain induced by trapezius inflammation

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The mechanism of the ectopic orofacial pain accompanied with the chronic neck pain remains unclear. We investigated the role of FKN in the ectopic orofacial pain following trapezius inflammation. CFA was injected into the trapezius in rats. We measured the head withdrawal threshold (HWT) to mechanical stimulation of facial skin by von Frey filaments. Moreover, changes in HWTs were examined after i.c.m. administration of neutralizing fractalkine receptor antibody (anti-CX3CR1). The expressions of the Iba1 which is maker of microglia or fractalkine receptor (CX3CR1) positive cells were examined in trigeminal subnucleus caudalis (Vc) and upper cervical cord (C1-C2), immunohistochemically. We studied the effect of fractalkine on nocifensive behavior. HWT was significantly decreased, and the density of Iba1-positive cells was significantly increased by CFA injection into the trapezius. After CFA injection, i.c.m. administration of anti-CX3CR1 produced a complete recovery of reducing HWT, and the density of Iba1-positive cells was also significantly reduced. HWT was decreased and the density of Iba1-positive cells was increased with i.c.m. administration of FKN. CX3CR1 co-expressed with Iba1 in trapezius-inflamed rats. The protein expression of FKN, but not CX3CR1 significantly increased in Vc to C1-C2. The results suggest that up-regulation of FKN following trapezius inflammation regulates microglial activation in Vc and C1-C2, and the activated microglia is involved in mechanical allodynia.

(COI: No)

P3-202

Involvement of LPA receptors and phospholipases in LPA-evoked peripheral itch sensation of mice

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Lysophosphatidic acid (LPA) is a phospholipid which is well corelated to itch intensity of cholestatic patients with itch, while it was also reported to induce acute pain. We reported that LPA caused itch-related behaviors rather than pain-related behaviors by using a cheek injection model. We also showed the involvement of transient receptor potential ankyrin 1 (TRPA1) and vanilloid 1 (TRPV1) channels in LPA-induced itch *in vivo* and *in vitro*. In this study, we further examined the LPA-induced signaling using calcium imaging methods with mouse dorsal root ganglion (DRG) neurons by focusing on LPA receptors and phospholipases. We used inhibitors of LPA_1, LPA_3, and LPA_5 which were reported to be expressed in mouse DRG neurons. We found that inhibitors of the all 3 receptors and LPA_5 alone were effective to reduce the LPA-responding DRG neurons, suggesting that LPA_5 is involved in the LPA-induced signaling. The downstream signaling of LPA receptors is so complicated that we pharmacologically examined the involvement of phospholipases A_2, C and D. Since lipid production or some membrane lipid depletion by these lipases can activate TRPA1 and TRPV1. An inhibitor of phospholipase D (PLD) decreased the LPA-responding DRG neurons, suggesting that PLD activity is involved in the LPA-induced signaling. Taken together, we concluded that LPA_5 receptor activation and PLD activity were necessary for the LPA-induced itch signaling in DRG neurons in the upstream of the activation of TRPA1 and TRPV1.

(COI: No)

P3-203

Involvement of ASICs in isoproterenol induced ischemic cardiac pain rat model

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Aim: Isoproterenol (Iso) induces myocardial ischemia, however, the cardiac pain associated with Iso-induced myocardial ischemia has not been reported. We characterized Iso-induced myocardial ischemia in rat to clarify the involvement of acid-sensing ion channel (ASIC) 3 in ischemic cardiac pain.
Methods: Male SD rats (200-400 g) were injected with 50 mg/kg of Iso subcutaneously. The control group were administrated with saline. Heart tissues were stained with hematoxylin and eosin. The behavioral changes and c-Fos expression in intra-spinal cord neurons after Iso-injection were analyzed. Furthermore, effects of morphine (Mor, 1 mg/kg) and ASICs antagonist amiloride (Ami, 30 mg/kg) on behavioral responses and c-fos expression were examined.
Results: The heart tissues was infiltrated with inflammatory cells into the subendocardial tissues 24h after Iso-injection. Rats showed characteristic behaviors most frequently from 15 to 30 min after Iso-injection. The animals lay on their side or back with their head extended. Iso group expressed more c-Fos in intra-spinal cord neurons compared to control group significantly. Pre-treatment with Mor significantly reduced the frequency of characteristic behaviors and the number of c-Fos expressed neurons. Furthermore, Ami decreased the characteristic behaviors and the c-Fos expression significantly.
Conclusion: These results suggest that ASICs were involved in Iso-induced ischemic cardiac pain.

(COI: No)

P3-204

Serotonin-mediated modulation on the chemosensory activity of rat carotid body

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We previously reported that immunoreactivities for the serotonin (5-HT) biosynthetic enzyme, tryptophan hydroxylase 1, and 5-HT plasma membrane transporter, were localized in chemoreceptor glomus cells and perivascular sympathetic nerve fibers in the rat carotid body (CB). In the present study, we examined 5-HT-induced intracellular Ca^{2+} ($[Ca^{2+}]_i$) responses in glomus cells, as well as smooth muscle cells and pericytes in isolated CB blood vessels. In specimen of glomus cells, 5-HT did not change $[Ca^{2+}]_i$ in glomus cells during normoxia, whereas induced repetitive $[Ca^{2+}]_i$ increases during hypoxia. The frequency of hypoxia-induced $[Ca^{2+}]_i$ changes was enhanced in the presence of 5-HT. These results suggest that 5-HT from glomus cells may increase its own hypoxic chemosensitivity by autocrine-paracrine mechanism. In arteriole specimen, 5-HT did not change $[Ca^{2+}]_i$ in smooth muscle cells. However, 5-HT induced $[Ca^{2+}]_i$ increases in pericytes in capillary specimen, and this response was inhibited by the 5-HT2 receptor antagonist, ketanserin. These results suggests that 5-HT from sympathetic nerve fibers may reduce capillary blood flow in the CB in order to increase chemosensitivity. In conclusion, 5-HT may modulate the chemosensory activity of the CB via two different modulatory pathways.

(COI: No)

P3-205

Enhanced tonic GABA currents after peripheral nerve injury contribute to inhibition of neuronal activity and subsequent remodeling of medial lemniscal fibers in the somatosensory thalamus

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We previously reported that cut of the infraorbital nerve (IONC) recruited additional excitatory medial lemniscal fibers onto somatosensory thalamic neurons (VPM neurons) around 5 days after the operation. On the other hand, it is also reported that peripheral nerve injury induces remodeling in the central nervous system through changes of inhibitory GABA system. However, little is known about details of the postoperative change in inhibitory GABA currents onto VPM neurons after the IONC. Here we report that the IONC significantly inhibited the neuronal activity of VPM neurons in unanesthetized mice and markedly enhanced the amplitude of tonic GABA currents onto VPM neurons. Interestingly, potentiation of tonic GABA currents after the IONC occurred much earlier than the remodeling of lemniscal fibers onto VPM neurons and was selectively observed in VPM neurons that had multiple lemniscal fibers by the IONC. Moreover, we found that chronic infusion of tonic GABA agonist into the VPM of normal mice recruited additional lemniscal fibers onto VPM neurons, whereas lack of tonic GABA currents prevented the remodeling of lemniscal fibers onto VPM neurons after the IONC. These results provide the possibility that the reduction of the VPM neuronal activity through enhanced tonic GABA inhibition by the IONC drives the remodeling of lemniscal fibers on a VPM neuron.

(COI: No)

P3-206

Morphogenesis of the lateral line in the primitive fish *Polypterus*

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A basal actinopterygian fish *Polypterus* possesses ganoid/enamel scales on the surface of the body, which reminds us of a primitive fish. There are a wide variety of shapes in lateral line scale and its neuromast known and we thus investigate into the morphogenesis of the neuromast of the lateral line scale in *Polypterus* as a representative of primitive fish. In a 25mm long larva, the primitive scales mineralized from a rear part toward a front part in a row, where primitive papillae on the vascular bone were formed. SEM images of the surface of a 115mm long juvenile revealed that the lateral line scale has a few pores from which sense hairs on top of the sensory cells project. These pores were surrounded by the epidermises in a concentric fashion and they looked just like the neuromast in other actinopterygians. The neuromasts in *Polypterus*, however, were macroscopically seen where pigment cells gathered, which is different from zebrafish. In a 125mm long juvenile, the isopedin was accumulated and the ganoin on the surface seemed to get formed. Axons from the neuromasts innervated into the vascular cavity of the lateral line scale and they thus went through it to join the lateral line, which was located at the junction of the transverse and the horizontal septa. The behavior of the axons was also immunohistologically confirmed in a 14mm larva. Therefore, the neuromasts started to be observed in the epidermis on the surface of the lateral line scale in the young larva before mineralization occurred, and their axons penetrated the scale and they eventually connected with the lateral line in the juvenile.

(COI: No)

P3-207

The early elevation of hippocampal BDNF by exercise after stroke protects neurodegeneration

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Exercise in the early stage of poststroke has been shown to facilitate the recovery from cognitive dysfunction. We have showed that the recovery of spatial memory function was depend on the hippocampal level of brain-derived neurotrophic factor (BDNF). However, time dependent changes of BDNF and its neuroprotective effects were unknown. In the present study, we investigated chronological changes of BDNF by exercise and apoptotic cell damage after brain ischemia using multifocal cerebral ischemia model rat induced by microsphere (MS) injection. Treadmill exercise was started at 24 h (Early group) or 8 days (Late group) after MS injection for 7 days. BDNF concentration in transected hippocampus were measured at 6hrs., 1, 2, 4, 8 and 15 days after MS injection by ELISA. BDNF concentration was gradually elevated by exercise (Early group: 6 hrs: 2.9 ± 0.21, 8 days: 4.8 ± 0.97; Late group: 8 days: 2.6 ± 0.67, 15 days: 4.9 ± 0.72 pg/mg-protein) and decreased after the completion of exercise in both groups. In addition, neurons immunostained for activated caspase-3 were observed in the hippocampus, especially in dentate gyrus, and tended to decrease in Early group. Taken together with our previous study, these results suggest that the transient elevation of hippocampal BDNF by exercise in early stage might play a major role in neuroprotection after onset of cerebral multiple microemboli.

(COI: No)

P3-208

Spatiotemporal analysis of motor map reorganization after stroke

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Previous studies have shown that motor map reorganization after stroke plays important role for functional recovery. Despite many factors affecting the motor map, little is known as to how or when motor map reorganization occurs and rehabilitative therapy modifies it. In the present study, the spatiotemporal changes of motor map were investigated after stroke. Rats were assigned to either rehabilitative or non-rehabilitative groups. After 3 weeks training of reach test, stroke were induced by photothrombosis in caudal forelimb area (CFA). Rehabilitative therapy was carried out by continuing reach training for 4weeks. To investigate the spatiotemporal changes of motor cortex, intracortical microstimulation (ICMS) were performed chronologically. In ICMS study, almost complete destruction of CFA was confirmed. Rostral forelimb area (RFA) was significantly reduced as well as vibrissa and jaw area, although RFA was located away from the infarction. RFA size increased slowly and its recovery was accelerated by rehabilitation. On the other hand, jaw and vibrissa areas were recovered quickly compared with RFA and not affected by rehabilitation. Interestingly, RFA size was also decreased after muscimol (specific agonist of the GABAA receptor) injection into CFA in normal rats. These results indicate that RFA size depends on the neural activity of CFA, suggesting rehabilitation therapy may modulate neural input into RFA.

(COI: No)

P3-209

Proteomic analysis of the molecular basis of the ischemic tolerance in the rat hippocampus

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Ischemic tolerance (IT) is a phenomenon whereby pretreatment with a non-lethal period of ischemia protects neurons against a normally lethal ischemic event. The cellular mechanisms underlying IT have not been fully elucidated. In the present study, we studied the altered expression of proteins in the rat hippocampus subjected to non-lethal ischemia by proteomic approach. Male S.D. rats were subjected to 3 min of global ischemia or sham-operation induced by four-vessel occlusion method. At 3 days after 3 min of ischemia, the hippocampal CA1 region was dissected and divided into four subcellular fractions. Proteins contained in each four subcellular fractions were separated by 2-DE. The protein spots were detected by CBB or silver staining. Mass spectrometry (MS) analysis was performed on LCMS-IT-TOF (Shimadzu). MS/MS spectra were searched against the NCBI database using MASCOT search program. Two-DE and MS/MS analysis revealed that the expression level of 11 proteins including VCP, aconitase2, transketolase, tubulin, adenylate cyclase-associated protein, protein-L-isoaspartate O-methyltransferase, adenylate kinase 1, PBP1, cytochrome b-c1 complex subunit 2, ACAT, voltage dependent anion channel (VDAC) was different between rats subjected to 3 min of ischemia and sham-operation. Western blot analysis revealed that the expression level of mitochondrial aconitase and VDAC was decreased in the hippocampus of rats subjected to 3 min of ischemia compared to sham-operated rats. The present results suggest that 3 min of ischemia may cause alteration in mitochondrial function.

(COI: No)

P3-210

A novel nucleoside analogue (COA-Cl) exerts neuroprotective effects against stroke events

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2Cl-C.OXT-A (COA-Cl) is a novel synthesized adenosine analogue exerting angiogenetic activity through ERK1/2 activation. As a well-known kinase, ERK1/2 is also involved in the over-activated apoptotic process of neurological disorders, such as stroke. Therefore, we investigate the neuroprotective effects of COA-Cl on stroke events in this study. Both rat transient focal cerebral ischemia and autologous blood infusion models were used for this study. COA-Cl was intracerebroventricularly administrated either immediately after model making or delayed continuously. In ischemic stroke, COA-Cl reduced the infarct volume, decreased the number of TUNEL positive cells and improved neurological deficits. The level of pERK increased by the administration of COA-Cl in vitro, indicating that the neuroprotective effects of COA-Cl may be via ERK1/2 activation. In intracerebral hemorrhage (ICH) models, the rats in the COA-Cl group attenuated the sensorimotor deficits and reduced ICH-induced edema. Furthermore, both TUNEL positive cells and 8-OHdG positive cells were fewer around the hematoma of COA-Cl treated rats compared with ICH ones. In conclusion, COA-Cl exerts neuroprotection against both ischemic and hemorrhagic stroke via anti-apoptotic and anti-oxidative effects.

(COI: No)

P3-211

Effects of running exercise on motor function and dendritic plasticity after unilateral striatal hemorrhage in rats

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We investigated the effect of running exercise on motor functions, dendritic plasticity and growth promoting or inhibiting factors after intracerebral hemorrhage (ICH) in rats. Male Wistar rats (B.W.: 240-270 g) were injected with collagenase into the left striatum to induce ICH. Sham operated animals were injected with saline instead of collagenase. They were randomly assigned to sham-control (SC), sham-exericise (SE), ICH-control (IC), and ICH-exercise (IE). Exercise groups were forced to run on a treadmill at a speed of 9 m/min for 30 min/day between 4 and 14 days after surgery. Behavioral assessments were performed by using motor deficit score, beam walking test and cylinder test. At 15 days after surgery, rats were sacrificed and their brain were removed. Dendritic morphologies in the motor cortex (layer V) were analyzed by Golgi-Cox staining. Expression levels of TrkB, Nogo-A and ROCK in the motor cortex were analyzed by Western blotting. Motor functions of IE improved significantly compared with that of IC. IE had more branches and higer length of dendrite compared with IC. TrkB expression levels of IE incresaed compared with IC. Nogo-A and ROCK expression levels of IE decreased compared with IC. These results suggest that improvement of motor function by treadmill running after ICH may relate to dendritic plasticity by the action of both growth promoting factors (TrkB) and growth inhibiting factors (Nogo-A, ROCK).

(COI: No)

P3-212

Effects of motor skills training on sensorimotor function and AMPA receptor subunits after intracerebral hemorrhage in rats

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The purpose of this study was to investigate the effects of motor skills training on sensorimotor function and AMPA receptor subunits after intracerebral hemorrhage (ICH) in rats. Rats were induced ICH by injection of collagenase into the left striatum. They were randomly assigned to acrobatic training group (ICH+AT) and no training group (ICH). ICH+AT group trained acrobatic tasks for 28 days from 4 days after ICH. Sensorimotor function was assessed using limb placing and postural instability test. The mRNA expression of AMPA receptor subunits, GluR1, GluR2, GluR3 and GluR4, in bilateral sensorimotor cortex was analyzed using real-time PCR at 29 days after ICH. This work was supported by a Grant-in-Aid for Scientific Research from the Niigata University of Health and Welfare, a Japan Society for the Promotion of Science and Nagoya Gakuin University. ICH+AT group significantly improved sensorimotor function compared with ICH group. The GluR1-4 mRNA expression of ICH+AT group significantly increased than those of ICH group in the sensorimotor cortex ipsilateral to the ICH. These results suggest that motor skills training following ICH may promote sensorimotor functional recovery by upregulation of AMPA receptor subunits mRNA expression.

(COI: No)

P3-213

Effects of coffee aroma on the stress: analysis of behavior and gene expression in the mice

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Recently, it was demonstrated that gene expression in the brain was altered by coffee aroma. However, effects of the aroma on brain regions are not yet clear. In the present study, we analyzed changes in behavior and gene expression in the mouse midbrain. After water emersion stress for 24 h, mice were received coffee aroma for 90 min. In elevated plus-maze test, time spent on open arms in the group of coffee aroma after stress was significantly longer than that of stress, suggesting that the aroma has anti-depressive effects. Gene expression, measured by RT-PCR, of nerve growth factor receptor (*NGFR*) and activity regulated cytoskeletal-associated protein (*Arc*) in the group of coffee aroma after stress showed tendency of increment and no change was found in brain-derived neurotrophic factor (*BDNF*) and FBJ murine osteosarcoma viral oncogene homolog (*c-fos*). NGFR is known as a receptor of nerve growth factor which has anti-oxidative activity. Arc has been suggested to be involved in cytoskeletal organization and inhibit apoptosis. The present results suggest that anti-oxidative effects by an increase of NGFR in the midbrain may protect neurons from oxidative stress. Moreover, a possible inhibition of apoptosis by Arc may result in protection of neurons. Coffee aroma may have anti-anxiety and anti-stress effects. There are no conflicts of interest to declare. Supported by a grant (#24650506) from the Ministry of Education, Culture, Sports, Science and Technology, Japan to Masuo Y.

(COI: No)

P3-214

Examination of the change of IDO2 expression in the mouse under inflammatory condition

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It has been reported that the depression is related to inflammation. By the way, the serotonin is produced from tryptophan, while indolamine 2, 3 dioxygenase (IDO) is the enzyme which converts tryptophan into kynurenine. Although the local existence in the normal brain of IDO is still discussed, it has been reported that expression of IDO rises to several times in brain when inflammation happens. When IDO increase under the inflammatory condition, tryptophan would be converted into kynurenine, and production of serotonin would be decreased. Therefore, we supposed that depression would happen as a result of serotonin decrease. However, it is still unclear that which tissue express IDO1 and IDO2, a novel isoform indolamine 2, 3 dioxygenase, under normal condition, because that is different according to reports. We think that it is necessary to examine expression of IDO1 and IDO2 in normal tissue in detail at first. Next, we examine the expression of IDO1 and IDO2 in the mouse tissue under inflammatory condition. Now, we investigate the expression of IDO1 and IDO2, a novel isoform indolamine 2, 3 dioxygenase, in the mouse tissue which given lipopolysaccharide (LPS) or polyinosinic:polycytidylic acid (poly I:C, a toll-like receptor-3 agonist), and caused inflammation, using in situ hybridization and northern blot analysis. We cannot yet say clearly, but the expression of IDO2 also may change by inflammation. In this conference, we show the results and discuss the change of IDO expression in the mouse tissue under inflammatory condition.

(COI: No)

P3-215

Changing in sociality, learning ability, and neural activity induced by the juvenile isolation in mice

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Social isolation for the period from weaning to sexual maturity interferes with the development of the medial prefrontal cortex (mPFC)-dependent behaviors in mice. Also, psychological stress leads to activation of microglia in mPFC. Based on these findings, it is thought that the neural activity in mPFC of mice, which are isolated for two weeks immediately after weaning, is abnormal as a result of microglial activation caused by psychological stress. However, no direct evidence has been reported. In this study, therefore, we made the mice which are isolated for two weeks after weaning, and compared the social interaction and learning ability with those of control mice. Social interaction and learning ability of isolated mice were found to be lower than that of control mice. In control mice, the blockade of microglial activity did not change the neural activity in mPFC. In contrast, the blockade of microglial activity increased the neural activity in mPFC of isolated mice. And, after blocking ionotropic glutamate receptors, the blockade of microglial activity increased the neural activity in mPFC of isolated mice.

(COI: No)

P3-216

Analysis of neurotransmitters in the brain and behavioral abnormalities of offsprings from stressed mother in mice

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Previous studies have shown that giving to stress in pregnant mice increases autism and attention deficit hyperactivity disorder (ADHD)-like behaviors in offsprings. However, the mechanisms involved are still unknown. In this study, we examined whether or not the molecular expression in the brain and behavior of the offspring are altered by giving stress to the pregnant mice. All experiments were performed using male offspring that had been prenatally exposed to stress, and male offspring whose parents had been exposed to prenatal stress. We examined sociality and anxiety-like behavior of the offsprings by behavioral experiments. In addition, we have examined the molecular expression in the brain by immunostaining method. Anxiety-like behavior was not observed in the first and second generation of offsprings. However, the reduction of sociality and anxiety associated with obsessive-compulsive disorder was observed in the first and second generation of offsprings. From the results of immunostaining method, reduction in the number of cells that contain serotonin was observed in all mice. Furthermore, the increase in CRF expression level was found only in the first generation of offsprings.

(COI: No)

P3-217

DL-/PO-phosphatidylcholine restores restraint stress-induced depression-related behaviors and spatial memory impairment

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The present study investigated the effects of 1, 2-dilinoleoyl-*sn*- glycero-3-phosphocholine (DL-PC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (PO-PC) on depression-related behaviors and spatial memory impairment in mice subjected to restraint stress. The immobility time in forced-swim and tail-suspension tests for mice subjected to restraint stress was significantly longer than that for nonstressed control mice, and oral coadministration of DL-PC and PO-PC (DL-/PO-PC; DL-PC : PO-PC=1 : 1) shortened the prolonged immobility time in a dose (0.1-5 mg/kg)-dependent manner. In the water maze test, the retention latency for stressed mice was significantly longer than that for control mice and DL-/PO-PC (1 mg/kg, *per os*) reversed the prolonged latency to control levels. Phosphorylation of Akt and glycogen synthase kinase 3 β (GSK-3 β) in the hypothalamus of stressed mice was significantly reduced compared with that for control mice, and DL-/PO-PC (1 mg/kg, *per os*) recovered the reduced phosphorylation of Akt and GSK-3 β. The results of the present study indicate that DL-/PO-PC has the potential to ameliorate stress-induced depressionrelated behaviors and memory impairment, possibly by activating Akt and inhibiting GSK-3 β.

(COI: No)

P3-218

Time-dependent effect of viral infection during pregnancy on brain 5-HT content using poly I:C

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It is well known that virus infection during pregnancy induces malformation(s) to the fetus including fetal cataracts, cardiac defects and deafness. However, little is known about influence of maternal virus infection to fetal brain development. Recently, we have shown that viral infection during pregnancy impairs fetal serotonergic development which may affect emotional or psychological status on offspring (Ohkawara et al, Brain and development, *in press*). At the last meeting of The Japanese Association of Anatomists, we reported rough critical period in impairment of serotonergic system on offspring by maternal viral infection. Now, we performed a detailed analysis of critical period and found that impairment of serotonergic system depend on at least two critical periods of viral infection during pregnancy and neonatal period. An early period is between gestation day (GD) 5 to GD17 and a late period is between GD21 to postnatal day 5. We will discuss the reason why impairment of serotonergic system has two critical periods of viral infection.

(COI: No)

P3-219

Ex-vivo MRI morphometric analysis of gray matter volume alterations upon rat PTSD-model stress

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Post-traumatic stress disorder (PTSD) is a mental disorder that occurs after exposure to severe stress. Voxel-based morphometry (VBM) is a comprehensive gray matter volume analysis by normalizing with a standardized template on brain MRI images. Several VBM studies were carried out in PTSD patients; however, it is unclear whether the neurological change is caused by the stress or by the patient's genetic disposition. In order to verify a causal relationship between severe stress and atrophy, VBM analysis in rats upon PTSD-model stress was carried out. Rats (male, 7 weeks old) in the experimental group were exposed to Single Prolonged Stress (SPS); 1) immobilization for 2 hours, 2) forced swimming for 20 min, 3) ether anesthesia. The rats were fixed after 7days, and their brains with skulls were removed. 3D-T2WI animal brain MRI images were then acquired. Gray matter regions were segmented using the standardized anatomical template, and VBM analysis was performed using Statistical Parametrical Mapping (SPM) 8. As a result, significant atrophy was detected in the thalamus, and right visual cortex (SPS: n=17, Sham: n=15, uncorrected $P<0.001$). These results suggest that thalamus and visual systems might be involved in the severe stress response and pathogenesis of PTSD.

(COI: No)

P3-220

Optogenetic activation of dorsal raphe serotonergic neurons produces antidepressant-like effect in mice

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It is unclear whether the activation of 5-HT neurons in the dorsal raphe nucleus (DRN) per se is sufficient for the treatment of depression. Recently, optogenetics using light-activated ion channels or pumps makes possible the modulation of target neurons in which these tools are specifically expressed. Here, we constructed a lentiviral vector (LVV) that induces expression of a channel rhodopsin 2 variant, ChETA, or light-activated proton pump for optogenetic inhibition, eArchT3.0, conjugated with EYFP under the control of tryptophan hydroxylase (TPH) promoter. One week after the injection of LVV into DRN of mice, we observed many EYFP-expressing neurons merged with TPH immunostaining. In whole-cell recordings in acute brain slice, photostimulation evoked single action potentials in ChETA-EYFP expressing neurons and inhibited neuronal firing in eArchT3.0 expressing neurons. Furthermore, in vivo photoactivation of the mouse DRN 5-HT neurons decreased immobility duration in the tail suspension test, while it had no effect on the anxiety-like behaviors in the elevated plus maze or open filed tests. On the other hand, photoinhibition of the DRN 5-HT neurons had no effect on these behavioral tests. These results suggest that the activation of DRN 5-HT neurons is sufficient to elicit antidepressant-like behavior in mice.

(COI: No)

P3-221

GABAergic interneurons in the spinal cord are increased in a chick model of spina bifida aperta

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Spina bifida aperta (SBA) is a complex congenital disorder with different neurological complications such as spinal ataxia, paralysis of the legs, and a lack of bowel and bladder control. We developed a chick model of surgery-induced SBA that shows spinal ataxia after hatching. However, the underlying pathophysiological mechanisms of SBA remain largely elusive. In this study, we examined the changes in inhibitory interneurons, GABA, glutamate decarboxylase 67 (GAD67), parvalbumin (PV), calbindin-D28K (CB), and calretinin (CR) in the spinal cord of surgery-induced SBA chicks on embryonic day 18. An immunohistochemical analysis showed increased levels of GABA and its synthesizing enzyme, GAD67, throughout the gray matter of the spinal cord in SBA chicks compared to normal chicks. We also found increased calcium-binding interneurons, PV, CB, and CR in the spinal cord of SBA chicks. The overexpression of calcium-binding proteins in the SBA chicks may have occurred to attenuate injury-mediated calcium excitotoxicity. Furthermore, the overexpression of GABA may represent at least a partly regenerative process following spinal cord injury as GABA influences the cytodifferentiation of developing neurons. In conclusion, we detected an increased level of GABAergic inhibitory neurons in the spinal cord of SBA chicks. This increase may have altered the inhibitory functions and, subsequently, muscle innervation of these chicks.

(COI: No)

P3-222

Autoantibody mediated CNS myelin morphology in the acute phase of experimental autoimmune encephalomyelitis

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Multiple sclerosis (MS) is the most common chronic inflammatory demyelinating disease of the CNS. Demyelination and axonal damage are responsible for neurological deficits in MS. However, the mechanisms of demyelination and axonal damage have not been fully understood. To clarify the mechanism of demyelination in experimental autoimmune encephalomyelitis (EAE), we examined myelin morphology during the course of MOG35-55-induced EAE in the C57BL/6 mice. Osmium-maceration scanning electron microscopic (SEM) analysis displayed ultrastructural abnormalities of myelin structure in the white matter of the EAE spinal cord. In addition, abnormal morphology of myelin was observed at early stages of EAE. While infiltrating immune cells into the CNS were not observed in the spinal cord, anti-MOG autoantibody was observed in the CNS at this point. These observations suggest that anti-MOG antibody plays an important role in the pathogenesis at the acute stages of EAE.

(COI: No)

P3-223

Evaluation of Motor Cortex Myelination in Developmental White Matter Injury Model Rat by Electron Microscopy

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Perinatal hypoxia-ischemia (HI) causes developing white matter injury (DWMI), which induces neurodevelopmental disabilities. We previously established a DWMI model rat (HI in postnatal day 3) that shows the deficits of hindlimb motor function and motor coordination. Electrophysiological change in motor cortex (M1) by intracortical microstimulation and weaker staining of myelin basic protein in the area were observed in our model. In this study, we challenged to know whether myelination was changed in M1 by electron microscopy. The DWMI rats were perfused with the fixative of 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M cacodylate buffer, and the brain coronal sections were soaked into the fixative, and processed for electron microscopy (EM) including fixation with 2% osmium tetraoxide. Ultrathin section of the motor cortex of hindlimb area were made and observed under EM. We counted the number of myelinated axons and their g-ratio in surface (layer II-III) and deep layer (layer V) of the cortex and corpus callosum (CC). No significant difference was observed in the number of both myelinated axons and its g-ratio in the layer II-III and the layer V. There was also no significant difference of those parameters in the CC. Data suggested that myelination in M1 is not related to the behavioral deficits in our DWMI model.

(COI: No)

P3-224

Analysis of oligodendrocytes in CD38 knockout mouse, a model associated with Autism Spectrum Disorder

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CD38, a type II transmembrane protein with ADP-ribosyl cyclase activity, is involved in Ca2+ -induced Ca2+ -release for Oxytocin (OXT) secretion in hypothalamic OXT neurons. In CD38 knockout (KO) mouse, a model associated with Autism Spectrum Disorder (ASD), decreased level of central Oxytocin secretion causes impaired social behavior, such as social recognition, pair bonding and maternal behavior. In this study, we investigated effect of CD38 on development of neurons and glial cells using CD38 KO mice from postnatal day 7 to 70. We found that MBP, MAG and CNP mRNA, markers of oligodendrocytes, were significantly decreased at postnatal day 7 to 14 in the cerebral cortices of CD38 KO mice. Furthermore, we confirmed that MBP and CNP proteins were also significantly decreased at postnatal day 14 to 21 of CD38 KO mice by western blotting and immunohistochemistry. These results suggest that deletion of CD38 cause inhibition of oligodendrocyte development in the cerebral cortex.

(COI: No)

P3-225

Maturation processes of developing prefrontal cortex of normal and disease model mice

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Altered development of prefrontal cortex (PFC) circuitry caused by a combination of genetic and environmental factors may underlay phenotypes of developmental psychiatric disorders like autism and schizophrenia. To gain insights into a sequence of biological events during PFC maturation, we performed gene expression, morphological, and drug sensitivity analyses. Expression of oligodendrocyte/myelin genes and a gene encoding fast-spiking interneuron marker parvalbumin were dramatically increased between postnatal days 7 (P7) and P21, and peaked at P21 and P35, respectively. Appearance of parvalbumin-positive interneurons increased drastically when we analyzed transgenic mice that express GFP under the parvalbumin gene promoter. Measurement of extracellular glutamate using in vivo microdialysis after an administration of MK-801, an NMDA antagonist, into the mouse PFC found that most responsive period for MK-801 administration was at around P42 and suggested that maturation of PFC circuit may take place after P42. These data suggest that circuitry maturation is a result of coordinated biological processes including synapse formation, myelination, and interneuron maturation and that many biological processes may be affected by diverse genetic and environmental insults, each having different sensitivity and time window. We are now characterizing genetically modified animals for genes that are associated with autism and schizophrenia to determine how circuitry maturation patterns are altered in these mice.

(COI: No)

P3-226

Intrathecal injection of mesenchymal stem cells ameliorates neurodegeneration of spinocerebellar ataxia type 1 mice

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No effective treatments have been developed for spinocerebellar ataxia (SCA). However, some studies have shown that mesenchymal stem cells (MSCs) are partially effective for Lurcher mutant mouse, a spontaneous genetic mouse model with cerebellar ataxia. Here, we tested the usefulness of intrathecal injection of MSCs for the treatment of SCA1 transgenic mice (SCA1-Tg). We observed that MSCs greatly mitigate cerebellar neuronal disorganization seen in the SCA1-Tg. Although Purkinje cells (PCs) of 24-week-old SCA1-Tg display multi-layer arrangement, MSCs-injected SCA1-Tg at the similar age showed mono-layer PCs. Furthermore, MSCs suppressed atrophy of PC dendrites in the SCA1-Tg. Finally, rotarod tests revealed that progressive deficits in motor coordination were significantly suppressed in the MSC-treated SCA1-Tg. However, we have not yet identified the mechanisms by which MSCs successfully ameliorated the neurodegeneration seen in SCA1 mice. Since recent studies have indicated the beneficial effects of factors released from MSCs through paracrine-mediated actions, we tested the hypothesis that some unknown factors released from MSCs are sufficient to prevent neurodegeneration of SCA1 mice. This notion was proven to be true. Upon injection of MSC conditioned medium into the SCA1-Tg, the progress of the behavioral defects were markedly mitigated. These results indicate an availability of a cell-free therapeutic approach against the SCA1. There are no potential conflicts of interest in the content of this study.

(COI: No)

P3-227

Mesenchymal stem cell-conditioned medium reduces the peripheral pathology in spinocerebellar ataxia type 1-knockin mice

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Spinocerebellar ataxia (SCA) is a major neurodegenerative disorder, in which autosomal-dominantly inherited polyglutamine diseases are the most frequent types and are caused by the expansion of a CAG trinucleotide repeat in the coding region of causative genes. SCA type 1 (SCA1) is caused by ataxin-1 protein (ATXN1) with an abnormally expanded polyglutamine stretch and is characterized by neurodegeneration in the nervous system. Mesenchymal stem cell (MSC) are defined as multipotent progenitor cells that can differentiate into mesenchymal lineage cells, such as osteoblasts, adipocytes, and chondrocytes, and into other cell lineages, such as glial cells and hepatocytes. We previously verified that MSCs reduce neurodegeneration seen in cerebellar Purkinje cell-specific SCA1-transgenic mice. Because MSCs are known to secrete a variety of growth factors that have both paracrine and autocrine activities in the damaged brain, we tested if MSC-conditioned medium can prevent the spinal motor neurons from neurodegeneration in SCA1-knockin mice. Application of the conditioned medium suppressed the neuro degeneration and consequently, maintained the conduction velocity in the spinal motor neurons of SCA1-knockin mice. These results suggest that unknown factors released from MSC mitigate progress of the functional disturbances seen in SCA1 knock-in mice in a paracrine manner. There are no potential conflicts of interest in the content of this study.

(COI: No)

P3-228

Cytoarchitecture of the cerebellum in the *laggard* mutant mouse

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The *laggard* mutant mouse, characterized by hypomyelination and cerebellar ataxia, is a spontaneously occurring mutant mouse caused by mutation in Kif14 (Fujikura et al., 2013). In this mutant, the laminated structures are cytoarchitecturally abnormal. Macroscopically, the cerebellum of this mutant mouse is smaller in size than the normal counterpart. Hematoxylin-eosin staining reveals that the mutant cerebellum has conserved foliation pattern, although they are rudimentary and the depths of the folds are markedly reduced. The lamination of the mutant cerebellar cortex is normal in general, but detailed analysis has demonstrated that granule cell layers are dramatically reduced, especially the internal granule cell layer that is almost absent. In the mutant, the Purkinje cell layer is cytoarchitecturally disorganized and arranged in a multiple cell layer instead of being arranged in a single line. The dendritic harborization of Purkinje cells is severely underdeveloped, as revealed by anti-calbindin immunostaining. TUNEL-positive cells in the external granule cell layer are increased in number, suggesting that the decreased population of granule cells in laggard mutant mouse is caused by the increased apoptotic cell death. In conclusion, the cerebellum of laggard mutant mouse is cytoarchitecturally affected, suggesting that the causal gene for the laggard mutation has multiple effects on the development of the laminated structures in the central nervous system in addition to the myelin formation.

(COI: No)

P3-229

Localization of huntingtin-associated protein 1-immunoreactive stigmoid bodies in the spinal cord of adult rat

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Huntingtin-associated protein 1 (HAP1) is a neural huntingtin interactor and is considered to be a determinant marker of the stigmoid body (STB). STB/HAP1 has putative protective functions against neurodegeneration. Although the expression of STB/HAP1 has been well described in the brain, little is known about its localization in the spinal cord. We immunohistochemically determined the distribution of STB/HAP1 in the spinal cord of adult Wistar rats in light (and fluorescence) and electron microscopy. HAP1-ir cells were abundantly expressed in the lamina I, II, III, V, sympathetic and parasympathetic preganglionic neurons of lamina VII, and lamina X, whereas HAP1-ir cells were relatively sparse in lamina IV and VI. In contrast, no HAP1-ir cells were found in the motoneurons of the lamina IX. Our present study suggests that STB/HAP1 in the spinal cord might play an important role in diverse spinal sensory and autonomic functions. Sensory and autonomic neurons in the spinal cord should be stable against stressful conditions as inducing neurodegeneration due to putative STB/HAP1 protectivity, whereas the motoneurons might be vulnerable to such stresses due to the absence of STB/HAP1 in lamina IX. Current results might explain why the spinal motoneurons are the constant target in certain neurodegenerative diseases.

(COI: No)

P3-230

Hyperphosphorylation of Tau at Ser396 occurs in the much earlier stage than appearance of learning and memory disorders in 5XFAD mice

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The present study investigated the relation of age-dependent spatial learning/memory impairment and Tau phosphorylation in 5XFAD mice, a model of Alzheimer's disease. In the water maze test, the acquisition and retention latencies for 5XFAD mice at 6 months, but not 2 months, of age was significantly longer than those for wild-type mice at the same months of age, without difference in the swim speed and visual acuity between two groups. The level of glycogen synthase kinase 3β (GSK-3β) phosphorylation at Ser9 in the hippocampus for the 5XFAD mice at >4 months of age was significantly lesser than that for wild-type mice at the same months of age, while a robust increase in the Tyr216 phosphorylation of GSK-3β was found both in wild-type and 5XFAD mice at 6 months of age, without no significant difference in the extent between two groups. There was no significant difference in the Tau phosphorylation at Ser202/Thr205 in the hippocampus between two groups, but Ser396 phosphorylation for 5XFAD mice was significantly higher than that for wild-type mice at ages ranging from 2 to 6 months. The results of the present study indicate that Tau hyperphosphorylation in the brain for 5XFAD mice precedes high activation of GSK-3β and occurs in the much earlier stage than appearance of learning and memory disorders.

(COI: No)

P3-231

Whale meat extract improves learning memory in Alzheimer's disease model mouse

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Introduction: Whale meat extract (WE) is known to strong anti-fatigue effects. Recently, we discovered that whale meat also have robust anti-oxidative effects. However the effects on the central nervous system, remain unclear. The Senescence-accelerated prone 8 (SAMP8) strain has been characterized by accelerated impairment of learning and memory, as used model mouse of Alzheimer's disease. We hypothesize the anti-oxidative effect of WE could affect on the prevention of pathogenesis of Alzheimer's disease.

Material & Method: In the present study we investigated the effects of whale meat extract, chronically administrated in the diet, and examine the behavioral test in the SAMP8 mouse.24-weeks age SAMP8 mouse fed the low-safflower oil diet (LSO) as control diet, or whale meet extract containing diet (WE) for 30 weeks. Then, we have investigated behavioral test to examine learning and memory including open-field test, Y-maze test, new object recognition test, and multiple-T-maze test.

Results: The WE group was significantly improved the learning memory in Y-maze test and multiple-T-maze test compared to LSO group.

Discussion: Our data revealed that whale meat extract improved the learning memory in Alzheimer's disease model mouse. These results suggest that the continuous eating of whale meat is able to prevent the dementia including Alzheimer's disease and senile dementia.

(COI: Properly Declared)

P3-232

Differential expression of alpha-synuclein with a cell-type dependent manner in vivo

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α-Synuclein is physiologically localized at presynapses and is also known as a major pathological component of synucleinopathies including Parkinson's disease and dementia with Lewy bodies. However, the precise relationship between the physiological functions and the pathogenicity of α-synuclein remains to be elucidated. To address this issue, we investigated the subcellular localization and the expression property of the protein. Recently, we reported that α-synuclein is localized at excitatory synapses, but not at inhibitory synapses in the hippocampus. There was a differential expression of α-synuclein between excitatory and inhibitory neurons. Here, we further investigated the expression profile of α-synuclein in the mouse whole brain. Localization of α-synuclein was similar to that of vesicular glutamate transporter-1. However, the protein was exceptionally colocalized with glutamic acid decarboxylase in the external plexiform layer (EPL) of olfactory bulb, lateral globus pallidus (LGP), medial globus pallidus (MGP), and substantia nigra pars reticulata (SNR). Now, we further study the relationship between the expression of α-synuclein and each neuronal cell-type. Regulation mechanism of the protein expression will be discussed.

(COI: No)

P3-233

Involvement of excess neuronal nitric oxide synthase in the dopaminergic neurodegeneration in Zitter rats

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Excess nitric oxide (NO) results in the formation of toxic peroxynitrite and causes neurodegeneration. Moreover, inhibition of inducible NO synthase forming NO in glial cells has been shown to display neuroprotective effects. However, little is known whether neuronal NO synthase (nNOS) forming NO in neurons affects the neurodegeneration. Here we focused on Zitter rat (Zi) which is autosomal recessive mutant rat and shows the dopaminergic neurodegeneration with age. Using the mutant rats, we investigated the distribution of nNOS in the nigrostriatal dopaminergic system and the effect of chronic administration of nNOS inhibitor, 7-nitroindazole (7-NI), on the neurodegeneration. The high levels of nNOS-expression were observed in the basal brain and midbrain of Zi. Furthermore, the increased number of nNOS immunoreactive (nNOS-ir) cells was observed in the substantia nigra pars compacta (SNc). In addition, chronic 7-NI administration significantly prevents the reduction of TH-ir cells in the SNc and fibers in the caudate putamen (CPU). Using HPLC reveals the protection of 7-NI against the reduction of dopamine in the CPU of Zi. These results indicate that excess nNOS is involved in the slowly progressive dopaminergic neurodegeneration in Zi, and the inhibition of nNOS prevents the degeneration.

(COI: No)

P3-234

Regulation of tau phosphorylation at the Alzheimer-specific AT100 sites by tau phosphorylation protein complex

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Tau, a microtubule-associated protein, is essential to the integrity of microtubules in neuronal tissues. Interestingly abnormally hyperphosphorylated tau is found as a characteristic component of paired helical filaments and neurofibrillary tangles in Alzheimer's disease (AD) brain. It has been speculated that hyperphosphorylated tau dissociates from microtubules, impairs microtubule (MT) function and then induces neuronal cell death. However a key determinant of abnormal phosphorylation of tau in AD is still to be clarified.

We have been proposing that degree of tau phosphorylation in neuronal cells is regulated by not kinases or phosphatase but the functional unit associated with MT as tau phosphorylation protein complex (TPPC). Multiple tau kinases, CDK5, GSK3beta, PKA, and the chaperons like 14-3-3 protein are the major participants of TPPC.

We focused on kinase-kinase activities on tau phosphorylation by the members of TPPC and conducted *in vitro* kinase assay by multiple combinations of TPPC-kinases (CDK5, GSK3beta, PKA). We found that phosphorylation of AT100 specific sites by PKA were enhanced with preincubation of CDK5 or GSK3beta. The simultaneous applications also induced enhancement of phosphorylation of AT100-specific sites. These results suggest achievement of phosphorylation on AT100-specific sites may the key regulation on abnormal phosphorylation of AD.

(COI: No)

P3-235

Neuronal damage after bone marrow transplantation in a mouse model of Krabbe disease

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Bone marrow transplantation (BMT) and umbilical cord blood transplantation are the only therapies available to date for Krabbe's disease (globoid cell leukodystrophy). BMT cross-corrects the activity of galactocerebrosidase, the missing enzyme in myelinating cells, via donor-derived monocytes that enter the nervous system. However, BMT does not cure the disease. To determine why BMT is not curative in the long run, we investigated the pathology of twitcher mice (twi), a model of Krabbe's disease, that lived over 200 days after BMT. Besides severe demyelination both in the central and peripheral nervous systems long-term after BMT, neurons appeared to be affected as evidenced by 1) sporadic axonal spheroids present in the spinal white matter and 2) bilateral defect in neurons of the ventrolateral thalamic nuclei. This study demonstrates that enzyme replacement by BMT is not sufficient in the long term. It will be necessary to supplement BMT perhaps with other interventions such as transplantation of glial progenitors and gene therapies.

(COI: No)

P3-236

Synaptic dysfunction and abnormal social behavior in mice with knockdown of autism susceptibility genes in the prefrontal cortex

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Autism spectrum disorder (ASD) is characterized by deficits in social interaction and communication, and stereotyped and repetitive behaviors. Recent studies have identified hundreds of genes whose mutations are found in patients with ASD. However, roles of such ASD susceptibility genes in synapse development/function and behavior, and brain regions in which they function remain largely unknown. We have developed an experimental system to examine the roles of ASD susceptibility genes in the development and function of synapses in the prefrontal cortex (PFC) and ASD-like behavior in mice. We performed RNAi knockdown of ASD susceptibility genes in pyramidal cells of the mouse PFC at embryonic day 14-15 by in utero electroporation. We examined contactin associated protein-like 2 (CNTNAP2) whose mutation is reported to be implicated in the development of ASD and whose knockout mice show ASD-like behavior (Penagarikano et al., Cell. 147, 235-246, 2011). We found that knockdown of CNTNAP2 decreased excitatory and inhibitory synaptic transmission in pyramidal cells of the PFC. Moreover, mice with CNTNAP2-knockdown in the PFC showed impaired social interaction and communication. These results clearly indicate that our system is useful for evaluating the contributions of ASD susceptibility genes to the development of synaptic function in the PFC and to ASD-like behavior in mice. Our preliminary data suggest that decreased excitatory synaptic transmission in the PFC may be related to ASD-like behavior.

(COI: No)

P3-237

Toll-like receptors control neuronal cell death via regulating the activation and production of nitric oxide in microglia cells of cathepsin D-deficient mice

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Cathepsin D (CD) deficiency induces ceroid-lipofuscin storage in lysosomes of mouse CNS neurons. We have previously reported that CD-deficient (CD−/−) mice die approximately postnatal day (P) 26 accompanied by not only intestinal necrosis but also neuronal degeneration. In addition, we showed that chemical inhibition of nitric oxide (NO) production reduces neuronal cell death. To understand the relationship of neuronal cell death and NO production, we investigated the role of Toll-like receptors (TLRs). For this, we generated triple knockout (tKO) mice of TLR2, TLR4 and CD. The tKO mice increased lifespan to approximately P 29. Although no difference was observed in aggregate formation positive for ubiquitin and p62/Sqstm1 in neurons, i) accumulation of activated microglia, ii) expression of iNOS in microglia, and iii) neuronal cell death were all delayed in the hippocampal pyramidal cell layer. These data suggest that the activation of microglia followed by the production of NO is triggered through TLRs.

(COI: No)

P3-238

Polarized localization of p62 and NBR1 regulates selective autophagy in cathepsin D-deficient neurons

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Cathepsin D (CD) deficiency is known to induce autophagy and accumulate abnormal lysosomes called granular osmiophilic deposits (GRODs) in mouse brain neurons that are often up-taken by autophagosomes. We show here that p62 and NBR1 are required for selective autophagy to eliminate GRODs. Immunocytochemistry and morphometry revealed that p62, NBR1 and ubiquitin were positive in neurons deficient in CD, while autophagosomes with GRODs disappeared from neurons deficient in CD, p62, and NBR1. Moreover, immunosignals of p62 and NBR1 were observed only in somatodendrites but not in axons of CD-deficient neurons, where no GRODs were detected. This localization pattern of p62 and NBR1 was confirmed in primary cultured cortical neurons. N-terminal specific domains of these proteins are responsible for the polarized localization. These results suggest that polarized localization of p62 and NBR1 regulates the degradation system through autophagy. This work is supported by JPSP KAKENHI Grant numbers 23110571, 23111004 and 25670099

(COI: No)

P3-239

Histological analysis of the brain in Dystonin-deficient mice

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Dystonia musculorum (*dt*) is an inherited mouse neuropathy characterized by progressive motor disorders. *Dystonin* (*Dst*) is a causative gene for *dt* mice. Although degeneration of the peripheral nervous system during early postnatal stage is well-recognized in *dt* mice, histological appearance in the central nervous system (CNS) responsible for motor disorders are still unclear. We generated a novel *Dst* gene trap mice, Dst^{Gt}, in which actin-binding domain-containing isoforms are disrupted. The gene trap allele encodes for a mutant Dst-LacZ fusion protein, which is detected by X-gal staining with high sensitivity. Homozygous mice showed typical *dt* phenotypes with progressive neurological symptoms, such as severe motor disorders in their limbs and twisted postures. Electrophysiological study showed abnormal co-contractions of agonist and antagonist muscles in Dst^{Gt} homozygotes. In histological analysis, abnormal neurofilament immunoreactivity was found in both somatosensory pathway and motor-related reticular nucleus, which showed high Dystonin protein expression. These results raise the possibility that cell-autonomous primary CNS defects contribute to *dt* phenotype.

(COI: No)

P3-240

Characterization of model mice for nuclear envelopathies

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Mutations encoding nuclear envelope proteins can cause variable human diseases including muscular dystrophy, cardiomyopathy with conduction defects, central and peripheral nervous system disorders, skeletal deformities, metabolic disorders, and premature aging, and they are so-called "nuclear envelopathy". Emery-Dreifuss muscular dystrophy (EDMD) is the firstly identified human nuclear enevlopathy, clinically characterized as muscular dystrophy, cardiomyopathy with conduction defects, and early joint contractures. Several causative genes for EDMD have been identified. Mutations in the *EMD* gene that encodes emerin, an inner nuclear envelope protein, cause X-linked EDMD and limb girdle muscular dystrophy (LGMD). Mutations in the *LMNA* gene, encoding A-type lamins of nuclear lamina, are known to cause various human diseases including autosomal forms of EDMD, LGMD, dilated cardiomyopathy with conduction defects, lipodystrophy, premature aging syndromes, and so on. We have previously produced emerin knockout mouse (EKO), that show mild conduction delay and locomotion abnormality. Lmna H222P knock-in mice (H222P) produced by the other group show severe cardiomyopathy. Here, we produced double mutant mice of EKO and H222P (EH), and found more severe skeletal muscle involvement. Surprisingly, cardiac muscle from EH mice show milder fibrosis compared to H222P mice. In this paper, we will show the gene expression profiles of each mutant mouse to consider tissue-specific roles of emerin-lamin A interaction.

(COI: No)

P3-241

Rbm24 is involved in tissue-specific aberrant splicing of Familial dysautonomia

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Tissue-specific alternative splicing is a pathway where complex networks of RNA-binding proteins (RBPs) are involved. The IKBKAP gene, whose mutation is found in almost all patients with Familial Dysautonomia (FD) exhibit aberrant splicing in neural tissue and fibroblasts, while others such as lymphoblasts and muscle tissue show a preference to normal splicing in the presence of the FD mutation. We were prompt to seek for tissue-specific regulators of FD aberrant splicing. We constructed a splicing reporter with the genomic region of the IKBKAP gene surrounding the FD mutation. We found this reporter recapitulates the abnormal splicing in fibroblasts of FD patients as well as HeLa cells. Using this bichromatic fluorescent reporter and cDNA library of RBPs, we found Rbm24 corrects exon-skipping caused by the FD mutation. Rbm24 functioned through an intronic sequence downstream of the FD mutation. RNA electromobility shift assays show that Rbm24 binds to and recruits U1 snRNP to this region and induces normal splicing in spite of the FD mutation. Since Rbm24 is expressed mainly in muscle tissue and scarcely expressed in neural tissue, we believe Rbm24 is involved in tissue-specific aberrant splicing of FD and possible therapeutic implications can assessed by its ectopic expression.

(COI: No)

P3-242

Schizophrenia brain elucidated by T1w/T2w ratio MRI

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MRI T1w/T2w ratio signal intensity (calculated by T1 weighted image signal intensity divided by that of T2 weighted image) cancels the receiver coil bias and increases the contrast related to myelin content. We used T1w/T2w ratio signal intensity to investigate whole-brain voxel-wise differences between 29 schizophrenia patients and 33 healthy controls and compared the results with those for T1w image. Normalized T1w/T2w ratio images were created for all the subjects and both gray matter (GM) and white matter (WM) components of T1w/T2w ratio image were smoothed with Gaussian kernels of 8mm full width at half maximum. Similar processes were also performed to T1w image. A two-sample t-test was undertaken using SPM8 with age and gender as nuisance covariates. Multiple comparison correction was performed by setting the family-wise error (FWE) at a threshold of $p < 0.01$. In GM we identified bilateral insula, right olfactory cortex, right putamen and right parahippocampal gyrus to be significantly decreased using the ratio image whereas only bilateral insula were found in T1w image. In WM we identified abnormal signal intensity in the right superior medial frontal white matter using the T1w/T2w ratio image while no regions were found in the T1w image. These results indicated that T1w/T2w ratio image enhances the pathological changes in the SZ brain due to reduced myelin contents and that it is useful to map the myelin-related changes in SZ brain.

(COI: No)

P3-243

The astrocyte-specific promoter for transgene expression in the marmoset brain

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Glial fibrillary acidic protein (GFAP) is the major intermediate filament protein specifically expressed in astrocytes. We examined a mouse and marmoset GFAP promoter region necessary and sufficient for the robust promoter strength and astrocyte specificity. At first, mouse GFAP promoter segments of 5 different sizes (1.9, 0.6, 0.3, 0.2 and 0.1 kb) were examined in their promoter strength and astrocyte specificity in mouse cerebellum in vivo, using lentiviral vectors expressing GFP under the control of one of the 5 mouse promoters. We found that the GFAP promoter of 0.6 kb in length showed robust promoter strength and astrocyte specificity, whereas the promoter of less than 0.3 kb was inferior to the promoter strength and/or cell specificity. Then, we cloned the GFAP promoter of 0.6 kb in size from marmoset genome, which showed in a mouse cerebellum a similar promoter strength with a mouse GFAP promoter of 0.6 kb and highly specific expression in astrocytes including Bergmann glia. Our results suggest that 0.6 kb of the GFAP promoter region is necessary and sufficient for the promoter strength and astrocyte specificity in mouse and presumably marmoset brains. We are examining properties of the marmoset GFAP promoter of 0.6 kb in marmoset brain.

(COI: No)

P3-244

Glia-tumor interaction in microenvironment of brain metastases

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Interaction between tumor cells and glial cells are important in the brain microenvironment. In the previous study, we observed that microglia and astrocytes accumulated around human lung cancer-derived (HARA-B) cells in a rodent model of brain metastasis. In vitro experiments showed that tumor cells and astrocytes stimulate each other by releasing cytokines. On the other hand, tumor cells were harmful for neurons. Interaction between tumor-microglia and astrocyte-microglia, or tripartite system of tumor-astrocyte-microglia remained elusive. In the present study, we investigated tumor-microglia interaction. We have found that tumor cell-derived factors suppressed microglial activation and the expression of antigen presentation. These results suggest that tumor cells and astrocytes stimulate each other but tumor cells suppressed immune responses induced by microglia, causing immune evasion.

(COI: No)

P3-245

Age-related changes in brain cytokine profile associated with enhanced recruitment of bone marrow-derived cells into the brain

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Neurodegeneration is associated with altered immune-brain interaction. The senescence-accelerated mouse prone 10 (SAMP10) is a model of early onset brain aging following immune senescence. We hypothesized that the brain-immune interaction is perturbed in SAMP10 mice. We created 4 groups of radiation chimeras by bone marrow transplantation using young and aged SAMP10 and B6 mice as recipients with 5-week-old GFP transgenic B6 mice as donors and analyzed chimeras immunohistochemically. Donor's marrow-derived cells of the myeloid lineage entered discrete brain regions through the attachments of choroid plexus. In chimeric mice with aged SAMP10 mice being used as recipients, larger numbers of marrow cells entered more brain regions than the other groups in the diencephalon. We performed multiplex cytokine assays to determine tissue concentration of 10 cytokines in the diencephalon prepared from young and aged SAMP10 and B6 mice. Aged SAMP10 mice exhibited higher concentrations of IL-6, G-CSF, CCL11, CXCL1 and CXCL10 than the other groups of chimera. Immunohistochemistry revealed that these cytokines were expressed in astrocytic processes of the attachments of choroid plexus, periventricular astrocytes, tanycytes, and hypothalamic neurons. Therefore, the enhanced recruitment of bone marrow-derived cells into the brain may be associated with region-specific changes in cytokine microenvironment in SAMP10 mice.

(COI: No)

P3-246

The cervical intramedullary sudomotor pathway speculated from the pathophysiology of hemifacial dyshidrosis caused by cervical disc hernia in humans

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To elucidate the cervical intramedullary sudomotor pathway, we analyzed 11 patients aged 37-74 years with hemifacial hyperhidrosis compensatory to the anhidrotic area caused by cervical disc hernia. Lesion estimation was performed via simultaneous qualitative sweat testing (Minor's method) and infrared thermography in an artificial climate chamber at 40℃ and 50% relative humidity. Neurological examination, and magnetic resonance imaging (MRI) were also performed. Hemilateral and segmental hyperhidrotic patterns were identified. MRI showed disc protrusion near the midline (median type) in the hemilateral sweat pattern, and approximately 3 mm lateral to the midline (paramedian type) in the segmental sweat pattern with no intramedullary lesion. In 80% of the latter, the disc protrusion was ipsilateral and corresponded to the anhidrotic segment. In the median type, the protruded disc may compress the central artery and cause insufficient peripheral perfusion of the sudomotor pathway around the anterior horn, causing ipsilateral anhidrosis without motor or sensory disorders. In the paramedian pattern, the disc may compress the sympathetic premotor neuron in the dorsolateral fasciculus, and spare the upper segments synapsing the spinal segmental autonomic interneurons and propriospinal neurons projecting to the intermediolateral nucleus. In conclusion, analysis of hemifacial hyperhidrosis could help clarify the cervical intramedullary sudomotor pathway.

(COI: No)

P3-247

Protective efficacy of the acupuncture stimulation for paclitaxel-induced peripheral neuropathy

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Paclitaxel (PTX) is a mitotic inhibitor used in cancer chemotherapy, but it develops chemotherapy-induced peripheral neuropathy (CIPN). Acupuncture is effective in the pathogenesis of pain. Therefore, we tested the influence of acupuncture stimulation (ACU) with PTX-CIPN model rats. The SD rats were randomly divided into 4 groups: PTX group, ACU of PTX pre-treatment (A-prePTX; ACU started on day 0), ACU of PTX post-treatment (A-postPTX; ACU started on day 14), and control group. All rats were injected intraperitoneally on 4 alternate days (days 1, 3, 5, and 7) with vehicle (saline) or 2.0 mg/kg PTX. Electro-ACU which caused slight muscle twitch was applied to ZuSanli acupoint (ST36) in the limbs on every other day (right side, 1Hz, 20 min., 3-5V). Behavioral assays were carried out by mechano-hypersensitivity von Frey hair test in the feet, sciatic nerve territory. All rats were sacrificed on day 35, and the lumbosacral spinal cord was collected for microscopy examination. PTX and A-postPTX group produced significant mechano-hypersensitivity in the feet, but A-prePTX group did not show any decrease in the mechanical threshold. In the PTX and A-postPTX, P2Y12 receptor a large amount of microglia appeared. In conclusion, our study indicates that the satellite cells in the dorsal horn of spinal cord cause PTX-CIPN. However, applying ACU stimulation before PTX administration relieves it. Therefore, ACU stimulation is effective in preventing PTX-CIPN, but any delay in the start of ACU treatment may decrease its effect.

(COI: No)

P3-248

Identification of novel target genes of mood stabilizer treatment

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Bipolar disorder (also known as manic-depression illness) is a severe and chronic psychiatric disease. The pathogenesis of bipolar disorder is not well understood. Medical treatments effect partially, and relapses are often experienced in spite of medications. There are needs for better therapeutics and elucidation of pathological mechanism. Some of antiepileptic drugs (mood stabilizers) have clinical efficacy in bipolar disorder, but there is a preliminary understanding of the mechanism of the treatment effect. Interestingly, mood stabilizers each have their own therapeutic properties. Among them, valproate (VPA) has preponderance of mania treatment, while lamotrigine (LTG) appears effective for prevention of depressive episodes but not manic. We hypothesized that VPA involves in the regulation of mania specific genes and LTG does in depression specific genes. To explore novel target genes of mood stabilizer treatment, we conducted a comprehensive analysis using microarray. We used C57BL6J mice for primary cerebral cell cultures. On the 10th day in vitro cultured cells were treated with 1mM VPA, 0.1mM LTG or control medium. We extracted total RNA from cultured cells. We analyzed gene expressions by microarray. 100 candidate genes were validated by quantitative real-time PCR, WB and immunohistochemistry. Finally we identified several specific genes regulated by VPA or LTG treatment.

(COI: No)

P3-249

Effect of systemic angiotensin II on exercise-enhanced neurogenesis in adult rat hippocampus

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Physical exercise enhances adult hippocampal neurogenesis via cell proliferation, which is promoted by direct action of growth factors on neuronal stem cells. However, the mechanisms between exercise and growth factor-dependent hippocampal neurogenesis are not yet fully understood. We found that exercise-enhanced hippocampal neurogenesis is cancelled by treatment of an angiotensin II (Ang II) type 1 receptor antagonist, losartan, suggesting that Ang II is involved in this enhancement. Here, we examined the role of systemic Ang II in exercise-induced hippocampal neurogenesis in adult rats. Plasma Ang II concentration increased rapidly in response to 30 min of treadmill running. After undertaking this exercise once daily for a week, the number of proliferating cells, identified by 5-bromo-2'-deoxyuridine (BrdU) incorporation, had increased approximately 1.5-fold in the hippocampus compared with controls. To mimic the increase in plasma Ang II concentrations brought about by exercise, rats were injected with 10^{-5} M Ang II once daily for a week. The number of BrdU-incorporating cells and of doublecortin-expressing newborn neurons, in the hippocampus rose approximately 1.5 and 1.9-fold compared with controls, respectively. The effects were completely abolished by losartan. These findings suggest that an increased levels of systemic Ang II during exercise may enhance neurogenesis in the adult rat hippocampus.

(COI: No)

P3-250

Neuroregenerative Effect of Conditioned Medium of Adipose-Derived Stem Cell on Cerebral Infarction in Mice

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Accumulating evidence has pointed that adipose-derived stem cell or its conditioned medium (ADSC-CM) might be available for neuroregeneration after cerebral infarction. The present study investigated the neuroregenerative effect of ADSC-CM and the underlying mechanism. We made a model of cerebral infarction by ligating the middle cerebral artery of CB17 mice. ADSC-CM or DMEM as a control was intravenously injected 1 h after infarction. Mice were sacrificed after periods of time, and the brain was removed and fixed for immunostaining with an anti-MAP2 antibody. Then, we measured the infarct volume and the ratio of the left hemisphere size relative to the right one (L/R ratio). We also carried out immunohistochemical analysis using antibodies against BrdU, Tuj1, and DCX, to evaluate regenerative neurons. There was no significant difference in the infarct volume between ADSM-CM and control groups. The L/R ratio for ADSM-CM group was significantly higher than that for control group ($P<0.05$), indicating that ADSM-CM alleviates atrophy of the ipsilateral hemisphere. Tuj1- and DCX-positive neurons were found along the border of infarction in ADSM-CM group, while little is detected in control group. Taken together, these results show that ADSC-CM has the potential to promote neuroregeneration and therefore, could be developed as a beneficial treatment of cerebral infarction.

(COI: No)

P3-251

Physiological analysis in *Drosophila* circadian pacemaker neurons with Ca^{2+}/pH-sensitive fluorescent proteins

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Since circadian oscillations in the concentration of cytoplasmic Ca^{2+} have been observed in the mammalian master clock suprachiasmatic nuclei as well as plants, intracellular Ca^{2+} may act as a messenger to link molecular clock oscillations with cellular physiological rhythms. However, in *Drosophila*, no circadian change in any ion concentrations in master clock lateral neurons (LNs) has reported. In this study, we first analyzed physiological activities in LNs in organotypic cultures of *Drosophila* central nervous system using Ca^{2+}-sensitive fluorescent protein Yellow Cameleon (YC2.1). As a result, we observed parallel circadian oscillations in both YC2.1 donor and acceptor fluorescence intensities, suggesting the possibility that cytosolic pH in LNs may oscillate in a circadian manner. To verify this hypothesis, we generated transgenic flies expressing ratiometric dual emission pH sensor deGFP4 and monitored intracellular pH in cultured LNs. As a result, we observed circadian pH oscillations in LNs. The pH values range from about 6.5 to 7.7 and the amplitude range of each oscillation were about 0.5 pH unit. In summary, LNs exhibited circadian oscillations in intracellular pH but not in intracellular Ca^{2+} concentration in the sensitivity range of YC2.1.

(COI: No)

P3-252

Oxtr expressed in GABA neurons at the MeA are suspected to control social memory

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OXT/OXTR system is well known as one of the regulating mechanism for social behavior, and thus OXTR deficient mice are considered as a useful model to study the neural mechanisms which govern social memory. We used this model to reveal regions and neuronal subtypes which control the social memories. In this study, we found that OXTR positive GABAergic neurons in Medial Amygdaloid nuclei (MeA) play an important role for constructing social memories. First, we found social stimulation induced neural activation in MeA. This finding implies that MeA contributes important role in constructing social memory. We next analyzed histologically to clarify neuronal subtypes of OXTR positive neurons in MeA. Interestingly, in situ hybridization analysis reveals that 30% of GABAergic neurons in MeA were expressing OXTR. It was acceptable because previous studies suggest that loss of GABAergic neurons causes dysfunction of social memory as same as OXTR deficient mice. Then we hypothesize these OXTR positive GABAergic neurons are necessary for social memory function. To test this hypothesis, we generated GABAergic neuron specific OXTR deficient mice by crossing loxP flanked OXTR mice and vesicular GABA transporter locus cre recombinase knocked in mice. This conditional knockout mouse showed social memory abnormality in our behavioral analysis. Taken together, these findings propose that OXTR positive GABAergic neurons in MeA play an important role in social memory, and it might be potential mechanism of pathology of mental disorder such as autism.

(COI: No)

P3-253

Coffee polyphenol chlorogenic acid protects neurons against glutamate neurotoxicity

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Stroke remains the leading cause of adult disability. Involvement of various neurotransmitters and neuromodulators have been shown to contribute to the ischemic damage and neuronal cell death associated with stroke. The roles of glutamate release, glutamate receptor-mediated Ca^{2+} influx, production of nitric oxide (NO) by activation of nitric oxide synthase, and oxidative stress in the pathogenesis of ischemic brain injury have been well established. Recent epidemiological studies suggested that moderate coffee consumption might reduce the risk of neurodegenerative diseases such as stroke. Coffee contains a larger amount of coffee polyphenol (chlorogenic acid) than caffeine, however, the roles of chlorogenic acid in the prevention of ischemic injury have not been fully examined. In the present study, we investigated the protective effects of chlorogenic acid on cell death using primary neuronal cultures of mouse cerebral cortex. Glutamate-induced neuronal cell death was inhibited by pretreatment with chlorogenic acid. On the other hand, there was little effect of chlorogenic acid on NO-induced cell death. Our results suggested that coffee polyphenol chlorogenic acid protects neurons from glutamate neurotoxicity. They provide a basis for the therapeutic target for ischemic stroke.

(COI: No)

P3-254

Transcriptional and post-translational regulation of transmembrane protein 132A

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Transmembrane protein 132A (TMEM132A) was first isolated from rat brain using PCR-selected cDNA subtraction, and it was found to be predominantly expressed in the brain. However, the transcriptional regulation of the TMEM132A gene has not been fully characterized. In this study, we characterized the promoter activity of the 880 bp region upstream of the mouse TMEM132A, identifying several putative sites recognized by transcription factors, which are highly conserved between the mouse and human TMEM132A genes. A mutational analysis of the TMEM132A promoter identified a critical region for its activation just upstream of the transcriptional start site. We also found that this region could be bound by the transcriptional factor MAZ, which overexpression resulted in downregulation of the TMEM132A promoter activity. Finally, we investigated the levels of TMEM132A mRNA and protein after exposure to five different neurotoxic stimuli, including thapsigargin, tunicamycin, serum starvation, homocysteine and hydrogen peroxide. Treatment with thapsigargin, a calcium modulating agent, markedly attenuated the levels of TMEM132A mRNA and protein in NSC-34 cells. These results give new insight into the mechanisms involved in regulating TMEM132 expression, and suggest that several transcriptional and post-transcriptional pathways regulate TMEM132A expression under developmental and pathophysiological conditions.

(COI: No)

P3-255

Neuroprotective effects of zonisamide against oxygen glucose deprivation

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Recently, zonisamide, a well-known antiepileptic drug, is also used in therapy for Parkinson's disease. However, roles of zonisamide in various aspects of neuroprotection mostly remains to be elucidated. Previously we demonstrated neuroprotective effects of zonisamide against oxygen glucose deprivation (OGD) using rat hippocampus slice culture and reported it at the 119th Annual Meeting of the Japanese Association of Anatomists. In this study, to analyze the molecular mechanisms of neuroprotective effects of zonisamide, we screened the genes, the expressions of which were changed by zonisamide, using microarray system. We prepared RNA from rat hippocampus slice cultures, which were assigned to 4 groups: control, zonisamide-administered, OGD-treated, and OGD-treated and zonisamide-administered groups (all groups were duplicated.). We performed gene expression profiling of the RNA samples using microarray system and compared the gene expression patterns among these groups. The results showed that among 40 genes which changed their expression more than 4 times by OGD treatment, 31 genes tended to be suppressed their changes by zonisamide-administration. These genes included inflammatory protein and cellular stress response protein. Furthermore, we detected so far unknown changes in several gene expressions induced by zonisamide alone. We will also discuss the functions of these genes in neuroprotection.

(COI: No)

P3-256

Differential effect of peripheral administered kainic acid on *vasopressin* and *oxytocin* mRNAs in the supraoptic and paraventricular nuclei of the hypothalamus

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The supraoptic (SON) and paraventricular nuclei (PVN) of the hypothalamus contain two types of magnocellular neurosecretory neurons: arginine oxytocin (OXT)-producing and vasopressin (AVP)-producing neurons. We have previously described that, by electrophysiological recording, kainite receptors (KARs) may be more highly expressed in OXT neurons than in AVP neurons in the SON neurons using transgenic rat lines. Here we examined the *in vivo* effect of kainic acid (KA) on OXT- and AVP-producing meurons in the SON and PVN using adult male Wistar rats. After 3h, 6h, 12h, 24h, 48h and 1 week after subcutaneous administration of saline or KA (4mg/kg), the gene expressions of the *OXT* and *AVP* in the SON and PVN were measured by in situ hybridization histochemistry. The gene expression of the *OXT* was significantly increased 3h, 6h, 12h and 24h after the administration of KA in the SON, magnocellular and parvocellular division of the PVN, and 48h after the administration of KA in the SON compared to saline, while, the gene expression of the *AVP* in the SON and the PVN did not differ among the groups. These results suggest that KARs are highly expressed in the OXT neurons in the SON and PVN, and that OXT neurons are more highly affected by peripheral administered KA than AVP neurons in the SON and PVN, which is consistent with our previous *ex vivo* study.

(COI: No)

P3-257

Selective retention of value representation in hippocampal CA1

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Hippocampus plays an important role in the formation of memories for space and events. However, it is not well understood how neuronal circuits are reorganized during the formation and retention of memory. To address this issue, we imaged neuronal activities during the learning using transgenic mice that express a fluorescent calcium sensor protein G-CaMP7 in hippocampal CA1 pyramidal neurons. Mice head-fixed under a two-photon microscope performed a memory task in virtual reality. Three target zones were placed along a virtual linear track. Mice need to remember correct target and stay there for 2 sec to receive reward. While the animals navigate through the track, a population of neurons exhibited place-cell like activity. In addition, another group of neurons fired while the mice stayed at the target zone. Over days, whereas the place-specific activities turned over to form new patterns, the representation of target was more stable: 2 % of all identified cells remained multiple days and still responded even reward zone were relocated. Therefore, these results indicate external representation has different stability in hippocampus. Information with higher value is more stably represented in subpopulations of pyramidal neurons in CA1 hippocampus than that without value.

(COI: Properly Declared)

P3-258

Total number of neurons of the hypoglossal nucleus after repeated crush injuries on the hypoglossal nerve in adult rats

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It is well known that peripheral nerve crush injury in neonatal animals causes retrograde neuronal cell death and thus results in drastic decrease in the total number of affected neurons. Although it is generally believed that this phenomenon does not occur in adult animals, a previous study reported substantial decreases in the total number of neurons of the hypoglossal (XII) nucleus by repeated crush injuries on the XII nerve in adult rats. Therefore, we re-examined the number of neurons in the XII nucleus after repeated XII nerve crush injuries by using stereological sampling, the most reliable counting method for whole quantification. Triple nerve crush injuries of the XII nerve were inflicted on adult rats at 1-week intervals, and the brainstem containing the XII nucleus was removed 4 weeks after the last crush. Frozen sections were cut at 50 μm, collected at 300 μm intervals, and stained with Nissl. The number of neurons in the XII nucleus was measured using an optical fractionator with the Stereo-Investigator software and total number of neurons in the XII nucleus was estimated. We report the effects of repeated XII nerve crush injuries on the number of XII neurons in adult rats.

(COI: No)

P3-259

Active participation of vasculature in immune-to-brain communication in the subfornical organ

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Inflammation in the body generates fever and activates the hypothalamic-pituitary-adrenal axis. Peripherally-released proinflammatory cytokines act on the brain and thereby cause these sick signs. Increased release of the cytokines into the general circulation can be experimentally achieved by systemic injections of lipopolysaccharide (LPS). Although LPS and cytokine cannot pass the blood-brain barrier (BBB), there are some small brain areas that lack a typical BBB, so-called circumventricular organs (CVOs). Among them, the subfornical organ (SFO) is a key site for immune-to-brain communication. Recently, we reported the occurrence of continuous angiogenesis in the CVOs of adult mice, suggesting that the vasculature of the CVOs has more dynamic property. In the present study, we showed that the administration of LPS decreased proliferation of endothelial cells and vascular permeability in the SFO. We focused on Platelet-Derived Growth Factor-B (PDGF-B) signaling in this system, because PDGF-B regulates vascular remodeling. After single LPS administration, PDGF-B protein levels increased in the SFO. Repeated LPS injection attenuated LPS-induced nuclear STAT3 translocation and c-Fos expression in the SFO. These data suggests that vascular remodeling in the SFO play an important role in immune-to-brain communication.

(COI: No)

P3-260

Distinct post-transcriptional regulation of monocarboxylate transporter 1 expression between neurons and non-neuronal cells in the adult mouse brain

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Rapid transport of monocarboxylates is essential for the carbohydrate, fat, amino acid metabolisms. The transport is facilitated by proton-linked monocarboxylate transporters (MCTs). In the present study, cellular expression of MCT1 in the adult mouse brain by fluorescent in situ hybridization and immunohistochemistry. In the hippocampal CA1, high neuronal expression was shown by intense MCT1 mRNA signals in pyramidal cells expressing vesicular glutamate transporter-1 (VGluT1) mRNA. Low expressions were also found for GABAergic interneurons expressing 67 kDa-glutamatic acid decarboxylase (GAD67) mRNA, astrocytes expressing plasmalemmal glutamate transporter GLAST mRNA, and capillary endothelial cells expressing vascular endothelial growth factor receptor-1 (VEGFR1) mRNA. By immunofluorescence, however, MCT1 immunoreactivity was intense in astrocytes expressing 3-phosphoglycerate dehydrogenase and capillary endothelial cells expressing glucose transporter-1, but negative in pyramidal cell dendrites and somata expressing microtubule-associated protein-2. Such a dissociated transcription and translational control in neurons was also found in Purkinje cells in the cerebellum and cholinergic neurons in the dorsal motor nucleus of vagus nerve. Therefore, neuronal expression of MCT1 is transcriptionally active, but suppressed at the post-transcription levels. Though this mechanism, predominant MCT1 expression in astrocytes and capillary endothelial cells is constructed in the adult brain.

(COI: No)

P3-261

Estrogen increases the expression of platelet-derived growth factor receptor alpha (PDGFRα) in NG2-positive oligodendrocyte precursor cells of the hypothalamus in rats

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NG2 cells are thought to differentiate into oligodendrocytes which have ability to maintain myelin structure. Therefore, they are known as oligodendrocyte precursor cells. However, their functions are no only limited as oligodendrocytes: it is suggested that they develop into neurons. It was also discovered that they express PDGFR α for their survival. In the present study, we examined whether estrogen induced PDGFR α expression in the rat hypothalamus. Firstly, we confirmed NG2 cells also expressed PDGFR α in the rat hypothalamus. Microglia (CD11b, Iba 1), astrocyte (GFAP), immature oligodendrocyte (O4), and mature oligodendrocyte (NS) did not co-localized in PDGFR α. This indicates that PDGFR α -positive cells are NG2 cells and oligodendrocyte precursor cells in the rar hypothalamus. Rats were ovariectomized and used for 2 weeks later. Estrogen treatment with a silicone tube implantation decreased body weight gain by decreasing the amount of food consumption. Also, this dose of estrogen was capable to increase the uterus weight. Importantly, estrogen treatment significantly increased the expression of PDGFR α protein in the hypothalamus revealed by western blotting. We suggest from the present study that estrogen induces PDGFR α signals in oligodendrocyte precursor cells and contributes to decrease in body weight controlled by the hypothalamus.

(COI: No)

P3-262

Regionalization of the Lamprey Telencephalon by *Foxg1*; Evolution of DV Patterning of the Vertebrate Telencephalon

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The telencephalon is the most complex and divergent structure of the brain. During development, the telencephalon can be divided dorsoventrally into a pallium (cortex) and a subpallium (basal ganglia). Regarding its regionalization, *Foxg1*, a downstream of FGF signaling, is thought to be a key mediator by suppression of *Wnt8b* in the pallium and promotion of subpallial fate by studies in mice and teleosts. However, the evolutionary history of the telencephalic DV patterning is less understood. Lamprey is only one of two living jawless vertebrates diverged from jawed vertebrates over 500 million years ago. In this study, we identified three *Foxg1* homologous genes from Japanese lamprey, (*L. japonicum*). Of those, *LjFoxg1b* is widely expressed in the lamprey telencephalon as in jawed vertebrates, whereas *LjFoxg1a* and *c* are expressed only in the subpallium. Functional assays utilizing FGF inhibitor resulted in significant reductions of *Foxg1* as well as subpallium markers (*Dlx* etc.) by contrast with expansion of pallium markers (*Wnt8*, *Pax6* etc.). We also identified three *Six3/6* homologous genes, required for the *Fgf8* induction at the anterior neural border in jawed vertebrates. The overall expressions of *LjSix3/6s* are similar with that of *Six3* and *6* in mice. Altogether, we point out that, those developmental mechanisms might have been established by the last common ancestor of vertebrates.

(COI: No)

P3-263

Reproducing Retinal Rod Bipolar Cell Light Response by Mathematical Model Including Neurotransmitter Receptors

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Electroretinogram (ERG) is clinically used for diagnoses of retinal diseases. However, detailed mechanism of the ERG wave has not been clarified yet. Therefore, it is useful to understand the quantitative physiological characteristics of the cellular functions of retinal neurons and the connections among these cells. In 1997, Ishihara et al. proposed a mathematical model of bipolar cell body. Although the model contains ion channel models, existing on cell body, neurotransmitter receptors, which are essential for reproducing the retinal light response are not included. Here, we propose a retinal rod bipolar cell model which can reproduce voltage response of light. This model is constructed by introducing two neurotransmitter receptor models, TRPM1 and GABA C receptor, and a simple amacrine cell model to the electrophysiological model of bipolar cell body proposed by Ishihara et al. TRPM1 channel functions as light signal source which is produced by photoreceptor cell, while GABA C receptor functions as lateral inhibition signal produced by the surrounding amacrine cells. Resulting action potential of the model were evaluated by providing several light signals, where experimentally obtained photoreceptor membrane potential shapes were used as input to TRPM1 channel. The resulting membrane potential shape showed good agreement with the experimentally obtained data. Additionally we considered to reproduce b-wave of ERG by using this model.

(COI: No)

P3-264

Effects of electrical microstimulation to the primate cerebellar dentate nucleus on the detection of stimulus omission in the missing oddball paradigm

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We recently found that neuronal activity in the dentate nucleus (DN) of the cerebellum exhibited temporally-specific firing modulation when monkeys performed the missing oddball task (Ohmae et al., J Neurosci, 2013). In this task, an audiovisual stimulus was presented repeatedly at a fixed interstimulus interval (ISI), then one stimulus in series was omitted. Monkeys were required to predict the timing of each next stimulus so as to make a saccade in response to the stimulus omission. We applied electrical microstimulation (200-333 Hz for 100-200 ms at 100 μA) to the DN 100 ms before the stimulus omission to test if neuronal activity played a role in temporal prediction. Electrical stimulation significantly shortened the reaction time by 67.8 ± 57.2 ms and 50.5 ± 73.3 ms (SD, n = 41, ISI = 400 ms, t-test, $p < 0.05$) for contraversive and ipsiversive saccades, respectively. The same stimulation current delivered before the second audiovisual stimulus in the sequence or during intertrial interval failed to evoke saccades, suggesting that neuronal activity in the DN might not simply represent saccadic motor commands. The effects of electrical stimulation varied depending on its timing during the ISI just before the oddball, indicating that the stimulation effects were modified by the existing neuronal activity. These results suggest that neurons in the DN may carry signals related to the prediction of stimulus timing that could be advanced by electrical stimulation.

(COI: No)

P3-265

Dose-related changes in hindbrain of prenatally X-irradiated rats

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Pregnant SD rats were exposed to a single whole body X-irradiation at 0.5, 1.0 or 1.5 Gy on gestational day 15, and their offspring at 4 weeks of age were intracardially perfused with 4% paraformaldehyde solution under deep anesthesia. T2-weighted MRI at 11.7-tesla was acquired from fixed brains, and then sagittal sections of the cerebellum and coronal sections of the brainstem were made. By MRI-based volumetry revealed dose-dependent reduction in the volume of cerebellar cortex. However, there were no alterations in the cerebellar lobulation and cortical cytoarchitectures of prenatally X-irradiated rats. Regarding the brainstem, immunohistochemical analysis was performed using anti-heat shock protein 25 (HSP25), a maker for cranial nerve motoneurons. In control rats, HSP25 immunostaining appeared in the motor and mesencephalic nuclei of the trigeminal nerve, facial nucleus, abducens nucleus, accessory facial nucleus, the magnocellular region of medial vestibular nucleus, the ambiguous nucleus, dorsal nucleus of vagus nerve, hypoglossus nucleus, the spinal tract of the trigeminal nerve, and facial nerve tracts. In prenatally X-irradiated rats, HSP25 staining in those neurons was enhanced with increasing doses of prenatal X-irradiation. The results suggest a dose-related cerebellar cortical hypoplasia and an increased expression of HSP25 in cranial nerve motoneurons and their related fiber tracts in prenatally X-irradiated rats.

(COI: No)

P3-266

The morphological differences between the right and the left cerebral hemispheres relating to the dominant hand observed with the naked eye and MRI

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The morphological differences between the right and the left hemispheres of the human brain have been remained to study. In the present study, using 25 brains for training of the medical students, in order to clarify the differences the following 2 points, which can be seen relatively easily with the naked eye, were observed with the naked eye and MRI: 1) the length from the frontal pole to the occipital pole (A-P), 2) the length from the cerebral longitudinal fissure to the most lateral part of the lateral surface of the hemisphere (M-L). Eighty-four % of all A-P cases were longer in the left hemisphere, about 80% of all M-L cases were longer in the right hemisphere. These results show that the left hemisphere is elongated anteroposteriorly and the right hemisphere is widened mediolaterally by the three dimensional pressure of the cranium and that the left hemisphere develops slightly earlier than the right hemisphere.
It is assumed that the earlier development of the left hemisphere relates to the right hand dominance. Observing MRI from the 7 left handed persons, it became clear that results for the above 1) and 2) cases in MRI was very similar to those of the naked eye observation. This means that the morphological differences between the bilaterality hemispheres do not correspond to the laterality of the dominant hand.

(COI: No)

P3-267

Structural changes in pericyte may cause the dysfunction of BBB in rat gliomas

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Blood-brain barrier;BBB is composed of endothelial cells, pericytes, astrocytes, and their basement membranes. Among them, pericytes have been attracting attention as one of the main contributors for construction and maintenance of BBB. Meanwhile, gliomas are characterized by angiogenesis of leaky blood vessels, which BBB is not properly functioned. We speculated that in gliomas, certain structural changes or scantiness of functional pericytes might be involved in the formation of disfunctional blood vessels and performed morphological examinations to elucidate the possible involvement of pericytes using a rat glioma model(RG2 glioma line). RG2 cells(1×106) were stereotactically injected in the right striatum of female Fischer 344 rat brains. After two weeks, animals were injected intravenously with tomato lectin in order to evaluate vessel structures and leakiness, and sacrificed by fixative perfusion. Glioma tissue sections were prepared for immunohistochemical examinations. Desmin+ and PDGFR β + pericytes were abundantly found on the leaky vessels characterized by extravasated lectin. Besides, they were covered by a type-IV collagen+ basement membrane together with endothelia similar to those in normal brain vessels. However, they typically showed various shapes, and projected multiple cytoplasmic processes into the stroma, which is not usually obserbed in normal brains. The new formation of dysfunctional vessels in gliomas might not be related not to scarceness of pericytes but to their certain phenotypic changes and dysfunction.

(COI: No)

P3-268

Visualization of cell division in neurons isolated from the rodent central nerveous system

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It has long been recognized that neurons in mammals complete cell division during embryonic and neonatal life and thereafter they differentiate and do not divide any more. However, current evidence shows that neural stem or progenitor cells exist in the postnatal brain and produce neurons *de novo*. The present time-lapse imaging shows that cultured rodent neurons are dividing. Cell division occurred in neurons derived from the various central nervous system (CNS) regions (cerebral cortex, hippocampus, hypothalamus and spinal cord) of rats at all ages from fetus to adult. Regardless of the CNS region and age, approximately 15% of neurons divided during 12 h and the mean division interval was 21 h. The divided cells were identified as neurons since they were positive for neuronal markers but not for glial markers, and showed action potentials. The cells identified as neurons by live-cell immunocytochemistry, expressing neuronal cell surface antigen Thy1.1 but not neuronal stem cell surface antigen prominin-1, were dividing. Cell division was also found in neurons of the Thy1-yellow fluorescent protein (YFP) transgenic mouse, which can be identified as neurons by fluorescence emission. The present study further indicated that some neurons in culture or immediately after isolation from new-born or adult rats were in cell cycle and showed mitotic figures, DNA precursor incorporation and DNA replication. These results suggest that rodent CNS cells showing neuronal characteristics have the ability of cell division under physiological culture conditions.

(COI: No)

P3-269

Distribution pattern of hydra synapsin revealed heterogeneity of synapses in the diffuse nervous system

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The diffuse nervous system (DNS) in cnidarians and ctenophores is composed of diffusely distributed and loosely connected neurons. The DNS is believed to be the most primitive form of the nervous system. Though the DNS appears to be homogenous neuronal network, conspicuous nerve bundles have been observed. These nerve bundles would be the phylogenetically oldest neuronal circuits. To understand the evolutional origin of neuronal circuits, we are screening for genes that are expressed in the nerve bundle around the mouth, the nerve ring, of Hydra. Among the candidate genes, we found that a Hydra gene homologous to synapsins in the other animals is expressed in the nerve ring. Hydra synapsin (HySyn) was generally detected as punctate staining along the neurites by immunostaining. At the ultrastructural level, HySyn immunoreacitivity was associated with synaptic vesicles. In addition to the nerve ring, a large number of HySyn-positive neurites were detected in the tentacles and the head region. In the other regions such as the body and peduncle, a small number of HySyn-positive fibers were observed. Our results indicate that HySyn is produced and utilized in the synapses of subpopulations of neurons in the Hydra DNS. Synapsin-expressing neurons in the DNS may have more elaborate synapses compared with the other neurons. The function of HySyn is currently being examined by in vivo transgenic approaches.

(COI: No)

P3-270

Autonomic nervous response and subjective sleep quality for sleep in older adults

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This study is to investigate the autonomic nervous response for 2 hours from onset of sleep and subjective sleep quality (SQ) for sleep in elderly The seven subjects aged 64-82 years participated in this study from three to six times during sleep, each in separated days. The subjects measured the ECG using heart rate monitor and the total sleep time (TST) using sensor mattress, while asleep and answered the questionnaires about SQ (VAS), the sleep onset, and so on. Autonomic function was estimated by the Lorenz plot and time domain analysis for RR interval (RRI). In this study, data were collected from 7 older adults over 21 nights in their own home. The TST and SQ in older adults were 363.5 ± 78.4 (min) and 67.9 ± 20.1. The mean RRI after the onset of the sleep first became longer, and then gradually became shorter, compared with during awake. The index of parasympathetic function (rMSSD) showed also a change similar to that of the RR interval. Our subjects showed that the SQ had positive significant correlation either with the TST ($r=0.610$, $p<0.01$), or with the sleep efficiency ($r=0.485$, $p<0, 05$). Number of nights lengthened in RRI was 16 of the all the experimental nights 21. In case of 16 nights, SQ showed positive significant correlations either with the TST or with the sleep efficiency. We concluded that although the SQ was influenced by the TST, it can be also related to other factors, such as sleep latency and number of awakenings. This work was supported by JSPS KAKENHI Grant Number 23593466.

(COI: No)

P3-271

Possible involvement of glucocorticoids in the inhibition by stress of sweet/umami receptor induction in rodents

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Chronic exposure to stress reportedly inhibits induction of common receptor (T1R3) for sweet and umami taste in rats. Here, we investigate whether endogenous glucocorticoids (GCs) are responsible for this receptor inhibition. In addition, mouse taste bud cells (TB cells) expressing T1R3 were used to examine the effect of exogenous GC on the induction of T1R3. Both adrenal glands were removed from rats [adrenalectomized (ADX) rats] and expression of T1R3 mRNA in fungiform papilae was examined by real time RT-PCR. The expression of T1R3 mRNA in the sham-ADX rats was significantly reduced in the ADX rats. The reduced mRNA expression was restored to the level seen in the sham-ADX rats by administration of the smallest dose (0.1 ng/kg, i.p.) of dexamethasone (DEX). However, the larger doses of DEX (10 and 1000 ng/kg, i.p.) conversely inhibited the enhancement of mRNA expression seen in the ADX rats given smallest dose of DEX. The mRNA expression for GC receptor (GR α) was detected in the mouse TB cells by RT-PCR. Significant reduction of T1R3 mRNA expression, as measured by real time RT-PCR, was observed in the TB cells at 24 or 48h after application of three doses of DEX (0.1, 1.0 and 10 μM). These results suggest that small dose of endogenous GC is necessary for the expression of T1R3, while the larger doses inhibit the expression, and that this inhibitory effect exerted by GC might be, at least in part, due to its direct action on the taste cells in rodents.

(COI: No)

P3-272

Regulatory role of AMPK in hypothalamic CRH neurons in social stress-induced alteration of feeding behavior

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Corticotropin releasing hormone (CRH) secreting neurons in the paraventricular nucleus of the hypothalamus (PVH) have important roles for the stress responses. Recently, we revealed that preferential activation of AMPK in CRH neurons in the PVH enhanced carbohydrate intake but not fat intake. This alteration of food selection behavior was caused by the activation of a subset of CRH neurons induced by AMPK activation. However, the physiological relevance of AMPK in stress induced alteration of feeding behavior has still been unclear. In the present study, the objective is to unravel whether AMPK in hypothalamic CRH neuron regulates stress-induced alteration of food selection behavior.

We subjected C57BL/6J mice to social defeat stress and examined the alteration of food selection behavior. To investigate the role of AMPK in the CRH neurons, we examined the effects of preferential expression of shRNA for AMPK in CRH neurons in the PVH. We constructed lenti virus vector expressing shRNA for AMPK in Cre recombinase (Cre) dependent manner, and injected into the PVH of CRH neuron-specific Cre expressing knock-in mice.

We found that social stress increased carbohydrate selection in C57BL/6J mice. This alteration of food selection behavior was completely blunted by expression of shRNA for AMPK in CRH neurons in the PVH. This result suggests that AMPK in CRH neurons in the PVH is important for stress-induced carbohydrate eating.

(COI: No)

P3-273

Influence of roller coaster boarding on human beings

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Purpose: The roller coaster of amusement park is popular. I evaluated the influence which roller coaster boarding has on the autonomic function, and considered the popular reason of the roller coaster.

Subject: Twelve young healthy male (23.5 ± 2.8years old). All the subjects never had ridden on the roller coaster which I used for loading. Eight subjects were fearful during boarding, and four were not. Ten subjects wanted to board once again, but two never wanted to board.

Method: I measured the plasma epinephrine concentration before and after roller coaster boarding. I recorded subject's holster ECG during roller coaster boarding and calculated HR. Sympathetic function (LF/HF) was calculated by using frequency analysis of RR intervals of ECG.

Results: 1. Plasma epinephrine concentration (1) Increase group (pre, post, No.) (40.2 ± 15.5, 58.5 ± 19.5, 4) (2) Slightly increase group (38.7 ± 16.6, 44.3 ± 17.5, 6) (3) No change group (63.5 ± 8.8, 62.1 ± 9.0, 2) 2. HR and Sympathetic function. (1) In epinephrine increase group, HR and LF/HF ratio increased (70 ± 20, 130 ± 25, 6) (3.5 ± 4.0, 20 ± 8.9, 6) (2) In epinephrine no change group, they did not change (80 ± 12, 110 ± 15, 6) (7.5 ± 5.8, 7.8 ± 7.5, 6)

Discussion: Six subjects whose epinephrine and LF/HF increased were considered to feel the fear of roller coaster as pleasure. Four subjects whose epinephrine slightly increased and LF/HF did not change were considered to feel the change of gravity as a pleasant sensation.

Conclusion: It was thought that there were two type roller coaster lover, those who like a fear feeling, and those who like gravity change.

(COI: No)

P3-274

Physical exercise reduces social avoidance induced by defeated stress

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Psycho-social stress is one of the important risk factor for depression. Recent studies reported that physical exercise eases depressive behavior. But, effect of exercise on psycho-social stress is unclear. In this study, we investigated the effect of exercise on social interaction behavior using social-defeat stress (SDS). Male C57BL/6J mice were attacked by retired ICR mice for 2.5 min. After the SDS, mice were transferred to another cage with (Ex) or without (St) freely accessible activity wheel for 2 hours. After then, mice were housed with the same ICR mouse resident preventing physical contact using acryl central plate during stress period. We also made control (Con) or exercise (N-Ex) group without SDS. The SDS was performed for 5 days followed by 2 days of no SDS. Two weeks later, we examined some behaviors in mice and monoamine level in brain. St group showed negative social interaction with ICR mice compared to Con group while Ex group did not show the negative social interaction. In contrast, N-Ex group showed positive social interaction. There were no significant differences in other behavioral tests among these groups. We observed that exercise decreased the elevated monoamine turnover by SDS in amygdala, which is critical site for fear memory. These results suggest that physical exercise reduces the SDS-induced social avoidance behavior with decrease of monoamine turnover in amygdala.

(COI: No)

P3-275

Effects of lactational perfluorooctanesulfonate exsposure on visual discrimination learning in adult male mouse offspring

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Perinatal perfluorooctanesulfonate (PFOS) exposure has been suspected to affect brain fuctions. However, little is known on the neurotoxic effects of PFOS on the cognitive functions. Here, we examined whether lactational exposure to PFOS affect the performance of visual discrimination learning and hippocampal development. For PFOS esposure, post pertum C57BL/6J mouse dams received 1 mg/kg b.w. of PFOS via gavage from post natal day 1 to 14. Control dams received water as a vehicle. After mice progeny reached adulthood, the visual discrimination learning task was conducted. After the completion of behavioral tests, extracellular amino acids levels in the dorsal hippocampus were assessed using *in vivo* microdialysis. The performance of PFOS-exposed group was significantly lower than that of control group. In addition, PFOS-exposed group showed higher extracellular glutamate levels in the dorsal hippocampus compared to those of the control group. These results suggest that lactational PFOS exposure affects learning ability and hippocampal development.

(COI: No)

P3-276

Peripheral clock gene expression by bright light exposure during daytime in humans

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Light is most strong synchronizer for control of circadian rhythm. The light intensity and the duration of light are changed through a year, affecting the body weight, food preference and melatonin secretion in humans and animals. We investigated that the effect of bright light exposure during daytime on clock gene expression using hair follicular and root cells. Seven healthy men participated in this study. The participants completed two 3-day experimental sessions in 1 month. The sessions consisted of a period of dim light on the first day, followed by a period of bright light exposure during daytime on the 2nd day instead of a bright light exposure. Other session consisted of a period of dim light exposure during daytime through the experiment for 3 days. Hair samples were taken at 3 pm, 6 pm, and 9 pm on the 2nd day and 3 am and 7 am on the 3rd day. We assessed mRNA changes in levels of *Per1, 2, 3, Cry1, 2, Rev-erb-α, Rev-erb-β, Dec1* using branched DNA probes. The clock gene expression of *Per 3* and *Rev-erb-β* were significantly increased through 3 pm and 7 am. However, the clock gene expressions were not significantly different between bright and dim light exposure in humans. It suggested that bright light exposure during daytime did not effect on the clock gene expression in humans.

(COI: No)

P3-277

Variations in endothelial function after mental stress during the menstrual cycle in young women

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The changes in the hormonal milieu throughout the menstrual cycle have direct actions on arterial wall physiology in women. However, very limited human data are available regarding the alterations in endothelial function at baseline and after mental stress during the discrete phases of the menstrual cycle. We examined whether the menstrual cycle influences the endothelial function after mental stress in young women. Female university students were tested during three phases of a normal menstrual cycle. Delineation of the three phases occurred as follows: (1) the early follicular phase; (2) the late follicular phase; (3) the middle luteal phase. Non-invasive measurement of peripheral endothelial function was determined by flow-mediated dilation (FMD) testing in the brachial artery during reactive hyperemia using echo and Doppler ultrasound. After the measurements of basal levels, they were subjected to mental stress evoked by the modified STROOP Color Word Test (CWT) in 10 min. The CWT induced arterial pressure (MAP) and heart rate (HR) elevations, and a slight vasoconstriction of brachial artery. The measurements of flow-mediated dilation (FMD) and maximal blood flow (%) were repeated at 5 min and 30 min after the CWT. Basal FMD varied during the three phases cyclically. In addition, the effect of CWT on FMD was changed during the menstrual cycle. These findings suggest the importance of menstrual phase in the interpretation of data on endothelial function.

(COI: No)

P3-278

5HT1A receptors in orexin neurons play an important role in regulation of REM sleep

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Orexin A and orexin B are lateral hypothalamic neuropeptides. A series of studies have suggested that orexin-deficiency causes narcolepsy in humans and other mammalian species, highlighting roles of this hypothalamic neuropeptide in the regulation of sleep and wakefulness. Orexins were shown to have a strong excitatory influence on serotonergic neurons in the raphe nuclei through both orexin 1 and orexin 2 receptors. Conversely, orexin neurons receive abundant input from the serotonergic neurons in the raphe nuclei. We also found serotonin potently inhibited orexin neurons through 5HT1A receptors, implying the negative feedback regulation. This linkage might play an important role in the regulation of sleep/wakefulness. To evaluate this hypothesis, we generated mice in which orexin neurons specifically lack expression of 5HT1A receptors utilizing Cre-loxP mediated deletion of 5HT1A gene. We examined sleep/wakefulness characteristics of these mice, and found that these mice exhibited several abnormality in sleep/wakefulness architecture. Also, these mice exhibited increase REM sleep amount after applying restraint stress. These observation suggests that serotonergic inhibitory regulation of orexin neurons play an important role in normal maintenance of sleep/wakefulness behavior.

(COI: No)

P3-279

A Single Nucleotide Polymorphism in the Human neuropeptides B/W receptor-1 Gene Affects Amygdala Function and Social Behavior

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Neuropeptides B/W recetptor-1 (NPBWR1) expressed specifically in lateral part of the CeA (CeL) plays a role in Amygdala function and social behavior. We found that a genetic variation of gene encodes a G-protein coupled receptor NPBWR1 might be one of the key factors that contribute to variation of social behavior in humans. As assessed by functional MRI, individuals carrying the loss-of-function SNP (404T>A) in the human NPBWR1 gene showed a stronger activation of the amygdala during passive viewing of faces expressing various emotions. On the other hand, we previously reported that NPBWR1-deficient (Npbwr1$^{-/-}$)mouse showed dramatic increase in social contact number in resident-intruder test, and decrease freezing behavior in contextual fear conditioning test. This time, we revealed that focal expression of hNPBWR1 gene in GABAergic neurons in the central amygdala in the Npbwr1$^{-/-}$; Gad67-Cre$^{+/-}$ mice reversed the abnormal social behavior of these mice, but that of hNPBWR1 with the SNP did not. Also, decreased freezing behavior in Npbwr1$^{-/-}$ recovered to the level comparable to WT mice with hNPBWR1 gene expression. These observations suggest that the SNP affects the function of hNPBWR1 and machinery that regulates amygdala function in response to various kind of social interaction in humans and mice.

(COI: No)

P3-280

Involvement of estrogen in the wheel running induced by oxytocin injection into the rat ventromedial hypothalamus

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Estrogen acts on the central nervous system and induces several behavioral changes. Some of those changes are mediated by the ventromedial nucleus of the hypothalamus (VMH). Estrogen is also known to facilitate wheel running. Our previous study has demonstrated that excitation of neurons in the rat VMH increase in wheel running. There exists oxytocin receptor (OTR) in the VMH and OTR is up-regulated by estrogen. Thus we hypothesized that OTR in the VMH regulate wheel running and estrogen modify this behavioral change. Microinjection of oxytocin into the rat VMH induced a dose-dependent increase in the wheel running. On the other hand, simultaneous injection of OTR antagonist, d(CH2)5-Tyr(Me)2-Orn8-Vasotocin inhibited the oxytocin-induced wheel running. Oxytocin administration into the VMH of ovariectomized (OVX) rats also increased in wheel running. In the estrogen-treated OVX rats oxytocin injection into the VMH further increased the running behavior than control OVX rats. Nocturnal wheel running in proestrus increased compared with other stage of estrus cycle. Injection of OTR antagonist into the VMH just before the onset of proestrus nocturnal period inhibited the following nocturnal wheel running. These findings suggest that oxytocin receptor in the VMH is involved in the induction of the wheel running. In female rats estrogen facilitate the effect of oxytocin on wheel running.

(COI: No)

P3-281

A new simple method for analysis of sleep by use of a subcutaneously implanted accelerometor in rats

Igarashi, Ayako; Omura, Natsumi; Miura, Megumi; Mima, Nanako; Nishimura, Yuri; Mabuchi, Kaori; Takamata, Akira (*Dept Environ Health, Nara Women Univ, Nara, Japan*)

Recording electroencephalogram (EEG) and electromyogram (EMG) is the standard method for evaluating sleep-wake state in rats. The wired EEG/EMG measurement restricts animal's activity, and telemetry system is relatively expensive. In addition, EEG/EMG-based methods are often unsuitable for use in high-throughput screens because they are time-consuming and involve invasive surgery. In the present study, we propose a new simple method for evaluation of sleep/wake using a subcutaneously implanted acceleration sensor. Rats were implanted with an acceleration sensor subcutaneously for measurement of the amount of activity every 2 min. We simultaneously measured the activity using an infrared sensor and also recorded EEG/EMG. The accelerometer can detect small movement more sensitively compared with the infrared sensor. Evaluation of sleep by the subcutaneous accelerometor after setting the threshold showed a good correlation with the evaluation of sleep using EEG/EMG, while the evaluation of sleep with the infrared sensor relatively overestimated sleep. Further, the assessment of sleep in multiple free-moving rats in the same cage can be performed with this method. Thus, this method could be employed for a screen for assessment of sleep.

(COI: No)

P3-282

Estradiol replacement attenuates osmoregulatory and angiotensin II-induced central body fluid regulatory responses in ovariectomized rats

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Estrogen replacement reportedly attenuates the drinking response to hypertonic saline infusion and i.c.v. angiotensin II (ANGII) administration in ovariectomized rats. In order to elucidate the site of the action of estradiol, we examined the effect of estradiol replacement on c-Fos expression in the central body fluid regulation-related sites in response to systemic hypertonic saline and i.c.v. ANGII administrations in ovariectomized rats. We also examined the ANGII type I (AT1) receptor expression in the hypothalamus by use of western blotting in estradiol replaced and not replaced ovariectomized rats. Estradiol replacement attenuated c-Fos expression at the organum vasclosam laminar terminalis (OVLT), supraoptic nucleus (SON) and paraventricular nucleus (PVN) in response to hypertonic saline infusion, and also attenuated c-Fos expression at the OVLT, subfornical organ, median preoptic nucleus, SON and PVN after i.c.v. ANGII administration. We also found that the hypothalamic AT1 receptor expression was less in estradiol-replaced rats than estrogen deficit rats. Our data suggest that estradiol possibly attenuates AT1 receptor expression in the hypothalamus, and the attenuated AT1 receptor expression may be involved in the attenuated body fluid regulatory responses to systemic hyperosmolality and central ANGII.

(COI: No)

P3-283

The effect of the mating and yeast ingestion on the salt ingestion behavior of *Drosophila*

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It have been reported that the fly show "specific hungers" for protein-rich food to produce eggs, and the mating augment egg-laying rate and food consumption. While protein ingestion or mating affect the subsequent intake of protein, it is not investigated whether the mating and protein intake elicit behavioral changes in salt intake. In this study, we measured intake volumes of salt (NaCl) solutions after mating and yeast ingestion. The mating and yeast ingestion caused males to reduce NaCl intake, on the other hand, females to consume increased amount of NaCl solutions. The inverse relationship between behavioral change of male and that of female led to consequent increase of female/male ratio of salt intake. The female/male ratio of 80 mM NaCl solution intake was 2.2 ± 0.9 in mated and yeast deprived flies, while 7.9 ± 0.9 in mated and yeast fed flies. It may be concluded that mating and yeast intake enhance sexually dimorphic intake of salt. It is interesting how the enhancement of sexual dimorphism of salt intake was induced. The decreased salt intake of male flies might be understandable since yeast contains salts and adult male flies do not require large amount of salt because they do not produce eggs. Female flies, by contrast, might require larger amount of salts than those contained in ingested yeast, because the egg laying rate might be increased by mating and yeast ingestion. The effect of mating and yeast ingestion on egg-laying rate should be investigated to confirm this assumption.

(COI: No)

P3-284

Modulatory mechanism of autonomic system that induced by acquired auditory experience in mice and rats

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Hearing a sound often elicits an emotion that is induced by not instinct but experience. In light of this sound effect, we tried to examine whether or not the hearing experience actually evokes emotional response using an experimental mouse model, in which mice were exposed to environmental sound stimulation coupled to different housing conditions. We exposed mice to an artificial sound stimulation under each of the pleasant (16 hours) and the unpleasant (8 hours) conditions in a day. After mice were spent in each of the two conditions for several days, we analyzed some physiological parameters of mice while exposing to a sequence of sound stimulations. Among the parameters, presenting a sound coupled to the pleasant housing condition significantly decreased hart beat rate. But in the model study have some unresolved issues, i.e., how long the autonomic effect remained, what evidence showed the mice feels pleasant or unpleasant, what is the difference point between this model and well-known conditioned reflex response. To resolve these question, we explored difference of signature of acquired brain function between the model mice and control mice with physiological, morphological and biochemical method. In this study, we tried to reveal the neural mechanism of this experience dependent autonomic nerve modulation. At first, we shut down the amygdala that involved in fears and some sound induced autonomic responses. Second, rats were checked by this model study, to get the full time autonomic response recording by telemetory apparatus.

(COI: No)

P3-285

Inhibitory effects of melatonin on age-related memory impairment in mice

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Deficits of learning and memory are one of the most striking phenomena of senescence. Recent studies have suggested that oxidative stresses are involved in the aging processes. Melatonin, a hormone secreted mainly by pineal gland has been well documented to have anti-oxidative effects. Melatonin secretion declines during aging, implying that the reduction of melatonin levels with age contributes to the aging process. The aim of this study is to investigate the anti-aging effects of melatonin on deficits in learning and memory during natural aging process. BALB/c mice were received long-term administration of either melatonin or vehicle in their drinking water from the age of 10 months (middle age) until 18 months (old age). We examined the effects of melatonin on spatial memory and object recognition memory using object location test and object recognition test, respectively, and on neurogenesis in the dentate gyrus (DG) of hippocampus and neuron sizes and numbers in the DG and the perirhinal cortex. Oxidative stress was measured by accumulation of 8-hydroxy-2'- deoxyguanosine (8-OHdG) in the hippocampal CA1 region and the peririhinal cortex. The data obtained in the present study suggested that the long-term administration of melatonin from middle age attenuates the age-related deficits of learning and memory via attenuation of age-related changes in neural structure and function, which would be mediated by melatonin at least in part through its anti-oxidantive effects.

(COI: No)

P3-286

Spatio-temporal dynamics of calcium activity in the cortex of naturally sleeping and awake mice

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Sleep is essential not only for the rest of body, but also for the maintenance of brain function. Electroencephalography (EEG), the summed activity of a large number of neurons in the cortex, shows that the slow wave activity (SWA) occurs during NREM sleep (also known as slow wave sleep). The SWA is presumed to reflect synchronous up and down states in cortical neurons and generate locally on a macro scale. However, it is unknown in what way sleep alters spontaneous activity of cortical individual neuron, especially their spatio-temporal pattern on a microscopic field. To address this question, we performed two-photon Ca^{2+} imaging of cortical neurons in naturally sleeping and awake mice. The mouse head was restrained with the head plate under the objective while the mouse could move limbs freely on the spherical treadmill. The mice were habituated to the experimental circumstances for five days. Using this method, we observed spontaneous calcium dynamics in the layer 2/3 of primary motor cortex during wakefulness, NREM sleep and REM sleep. The synchronicity of spontaneous calcium signals among neurons changed in response to the sleep stages. The synchronization did not depend on the distance between neurons up to micrometer-order. Our results, taken together with other studies, suggest that cortical activity synchronize regionally but not locally during sleep.

(COI: No)

P3-287

The pontomedullary tegmentum GABAergic neurons are involved in the regulation of sleep and wakefulness

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Sleep is regulated by several subcortical regions including the brainstem. However, the detailed locations and functions of the brainstem sleep-related neurons are incompletely identified. To explore novel neurons regulating sleep and wakefulness, we systematically manipulated firing of neurons in the pons and medulla oblongata, and investigate if their firing affects sleep. Region- and cell-type specific gene delivery was achieved by local injection of Cre-inducible AAV vectors into the GAD1-Cre mice. To silence their firing, the inhibitory DREADD hM4Di was targeted to them. Histological studies showed that 99.3% of hM4Di-expressing neurons were GABAergic. Patch-clamp recordings revealed that the hM4Di agonist CNO suppressed their spontaneous discharge. We discovered that a subset of pontomedullary tegmentum GABAergic neurons regulate sleep and wakefulness. Suppression of GABAergic neuron firing in the dorsal area of pontomedullary tegmentum increased the total amount of NREM sleep, whereas inhibition of the medial GABAergic neurons had no effect on sleep and wakefulness. These results suggest that GABAergic neurons in the dorsal area of pontomedullary tegmentum contribute to the regulation of sleep.

(COI: No)

P3-288

Modulation of masseter activity by vigilance states and circadian rhythm

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Bruxism is associated with an increase in the activities of the jaw-closing muscle; however, the alteration of the jaw-closing muscle activity among vigilance states is unclear. We examined the influences of dark/light and sleep/wake cycles on the activity of the masseter muscle in comparison to those of the neck muscle over a 24-h period in mice. The mean EMG activities of the masseter and neck muscles during wakefulness (W) were much larger than those during non-REM sleep (NREM) and REM sleep (REM). In contrast, the mean EMG activities of the masseter and neck muscles during W and NREM were significantly smaller during the transition period from dark to light. During NREM, the masseter EMG activity was moderately correlated with the neck EMG activity in both dark and light periods, whereas there was no correlation between two muscles during W or REM. During W and NREM, bimodal distributions were found in the masseter EMG activity, whereas the neck EMG activities were unimodal distributions in any state. These results suggest that the activities of the masseter and neck muscles are modulated by both sleep/wake and dark/light cycles. Furthermore, even during NREM, the masseter muscle is activated bimodally, which may contribute to the occurrence of raised masseter muscle activity such as sleep bruxism.

(COI: No)

P3-289

Circadian profiling of an interaction between BMAL1 and CLOCK by FRET bioimaging

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A circadian rhythm is a crucial factor in the regulation of a wide range of physiological processes that are involved in biological systems such as the endocrine system and the sleep-wake cycle. The systemic circadian system can be broken down into cellular rhythms, which are maintained by periodic change in the status of a set of clock genes and proteins. Such proteins oscillate not only at the levels of their expression but also at those of posttranslational modification.
FRET (Förster resonance energy transfer) is a phenomenon of radiationless energy transfer between a pair of fluorophores, where wavelengths of fluorescence emission are altered in a manner dependent on the distance between them. Therefore, in combination with a color pallet of different fluorescent proteins, it enables us to examine protein-protein interaction, protein conformational change, and enzymatic activity in living cells or organisms. In this study, we have constructed fluorescent biosensors for clock proteins, BMAL1 and CLOCK, and performed qualitative and quantitative observation of circadian rhythms of protein dynamics, including their subcellular localization, mobility, and interaction, in living cells.
(COI: No)

P3-290

Analysis of diurnal yawning rhythm in Wister rat

Ikegami, Keisuke; Shigeyoshi, Yasufumi (*Dept Anatomy Neurobiol, Kinki Univ Fac Med, Osaka, Japan*)

Yawning is known to have the important physiological function because yawning is observed in multiple species, not only mammal but also birds and reptilian. Yawning has been thought to be happen when we feel sleep, but resent studies suggest that yawning has the arousal effect. In addition, brain disease or stress may induce the yawning with typically arousal effect. However, the mechanism and physiological functions of yawning remain unclear. To clarify it, it is important to confirm the diurnal rhythm of yawning and the relationship with sleep-awake patterns. However it has been unknown in rat, which has been a nice model and contributed to the studies of yawning. In this study, we has performed the time-course monitoring of 10-weeks-old male Wister rat under individual and group housing. We suggest the time-dependent yawning pattern and the relationship with another rat.
(COI: Properly Declared)

P3-291

AVP-releasing rhythm of SCN and SON in culture

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In mammals, circadian rhythms are driven by a pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Recordings of neuronal firing of dissociated SCN cells suggest that single SCN cells are competent circadian oscillators. The SCN cells also show clear circadian rhythm of arginine vasopressin (AVP) release in cell culture when they are plated at high density. Both the amount of AVP release and amplitude of the rhythm depended on the plating density. Co-culture with cortex cells could not restore the loss of rhythmicity in low-density culture. AVP is also produced in the supraoptic nucleus (SON). However, SON did not show any circadian rhythm in culture. When SON cells were added to low-density cell cultures of SCN, the amount of AVP release, but not the rhythm amplitude was increased. These results suggest that SON cells do not show AVP-releasing rhythm even in the presence of rhythmic SCN cells.
(COI: No)

P3-292

Development of the animal model of shift work using the mouse

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In the shift work, there are a lot of health damage that are not only the acute such as complained of insomnia and decrease of alertness but also chronic risk such as hypertension and diabetes. The purpose of this study was to develop the animal model of shift work (SW) using a wild type mouse (C57BL/6) and we investigated the effect of the separation between biological rhythms and light-dark cycle on the animals. To create the SW mouse, we permitted the mouse to run on the running wheel and to eat the food during only light period and limited both of them in the dark period. In control group, we allow them during the dark period and permit them during the light period. After one week of baseline recording, body temperature, amount of spontaneous activity and running wheel activity were measured for two weeks. As a result, in SW group, the phase of body temperature, spontaneous activity and running wheel activity was shifted to the light phase. These results suggested that we successfully created an animal model of shift work as we observed the apparent separation between biological rhythms and light-dark cycle.
(COI: No)

P3-293

Insulin resistance in heart-specific *Bmal1* knockout mice

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The regulation of mammalian energy balance such as glucose and lipid metabolism is influenced by function of the circadian clock, which is composed of a set of core clock genes. Since functional clock genes are widespread throughout the body, diverse organs might participate in clock-controlled energy metabolism. However, the impact of altered clock function in specific organs on the regulation of glucose metabolism remains unclear. Here, we demonstrate that heart-specific disruption of the circadian clock gene *Bmal1* not only results in significant reduction in cardiac function but induces impaired glucose metabolism in mice. The glucose tolerance test showed that glucose tolerance was significantly impaired in heart-specific *Bmal1* knockout mice. In addition, the insulin tolerance test revealed a decrease in insulin sensitivity in the knockout mice, indicating that hyperglycemia observed in these animals was due to systemic insulin resistance. Although glucose metabolism may have been affected by increased body mass, body weight was not different between control and heart-specific *Bmal1* knockout animals. Our results suggest that functional clock in the heart is an important component of the circadian clock network that maintains mammalian glucose metabolism.
(COI: No)

P3-294

Aging Dissociates Circadian PER2 Oscillations of Individual Cells in the Suprachiasmatic Clock

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In aged C57BL/6J mice, decreased amplitude and increased fragmentation of the wheel running rhythm and lengthened circadian free-running period have been observed. Evidences indicating that aging impacts the neural activity rhythms in the suprachiasmatic nucleus (SCN) were reported by many laboratories. However, there are few reports that show circadian oscillations of clock genes in the SCN was disrupted by aging. To explore the contradiction between neural activity and clock gene rhythms in aged SCN clock, we have carried out ex vivo bioluminescence recordings from cultured SCN slice of young and aged PER2::LUC mice. As previous reports, there was little change in the amplitude of PER2::LUC rhythm between the young and aged SCN explants from animals that were housed in a normal light/dark condition. However, PER2::LUC rhythm in the aged SCN taken from animals that were housed in a constant dark condition for 10 days showed longer circadian period with lower amplitude. Results from recording PER2::LUC rhythm of SCN individual cells using the electron multiplying CCD camera, individual cells of aged SCN showed longer circadian period of PER2::LUC oscillation and desynchronization between individual cells. These data suggest that the molecular clocks in individual SCN cells are also degraded by aging.
(COI: No)

P3-295

Role of estrogen receptor β in the medial preoptic area in the regulation of aggressive behavior in male mice

Nakata, Mariko[1]; Sano, Kazuhiro[1]; Musatov, Sergei[2]; Yamaguchi, Naoko[3]; Sakamoto, Toshiro[4]; Ogawa, Sonoko[1] (*[1]Lab Behav Neuroendo, Univ Tsukuba, Ibaraki, Japan; [2]Weill Cornell Univ Med Col, USA; [3]Dept Med, Aich Medical Univ, Aich, Japan; [4]Dept Health Sci, Kyoto Tachibana Univ, Kyoto, Japan*)

The expression of male-type social behaviors such as sexual and aggressive behavior highly depends on the action of testosterone (T) in which T plays central role in both the facilitation of behaviors and the development of their neural bases. Moreover, T is known to activate estrogen receptors (ER) α and β after being aromatized to estradiol in the brain. We have shown that the activation of ERα in the medial preoptic area (MPOA) is absolutely necessary for the facilitation of male sexual, but not aggressive behavior (Sano et al. EJN, 2013). However, the contribution of ERβ in the MPOA to the regulation of male-type social behavior has yet to be determined. Thus in this study, we site-specifically knocked down ERβ in the MPOA and examined its effect on the expression of male sexual and aggressive behaviors. At the age of 21 days, gonadally intact male mice (ICR/Jcl) were bilaterally injected either with adeno-associated viral vector silencing ERβ or a control vector in the MPOA. Starting at the age of 12 weeks, all mice were tested for their sexual and aggressive behaviors. Surprisingly, knocking down of ERβ in the area reduced the expression of aggressive, but not sexual behavior. Our results suggest that ERα and ERβ in the MPOA may be responsible for the differential regulation of male sexual and aggressive behavior by testosterone.(KAKEN #23240057 to SO)

(COI: No)

P3-296

Monitoring of circadian rhythm in arginine vasopressin expression by a bioluminescence reporter

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Arginine vasopressin (AVP) is a major neuropeptide in the suprachiasmatic nucleus (SCN), where the master circadian pacemaker is located in mammals. AVP is a plausible transmitter of circadian signals from SCN to other areas, but the role of AVP in clock functions is not well understood. AVP is also expressed in the paraventricular nucleus (PVN) and supraoptic nucleus (SON). Due to a lack of analytical tool, dynamics of AVP production with high temporal and spatial resolution are not well understood. In the present study, we produced knock-in mice carrying an Emerald-luciferase reporter (AVP^{ELuc}) to monitor AVP expression in cultured brain slices. Bioluminescence (AVP::ELuc) was measured either from a whole tissue with a photomultiplier tube or from individual cells with an EM-CCD camera. SCN slices showed robust circadian rhythms for more than 10 days in AVP::ELuc bioluminescence. The peak phase of the rhythm was located at the middle of the day. AVP::ELuc bioluminescence in the PVN and SON exhibited a huge peak on the first day of culturing, thereafter, rhythms with significantly low amplitude persisted in the subsequent days. AVP^{ELuc} knock-in mice are useful not only for circadian but also for neuroendocrinological studies.

(COI: No)

P3-297

A GABAergic mechanism is indispensable for Per2-suppressing effect in the rat SCN by sevoflurane

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The inhalation anesthetic, sevoflurane, have the suppressive effect on the clock gene *Per2* expression in the rat suprachiasmatic nucleus (SCN). We examined intra-SCN spatial susceptibility to sevoflurane and the involvement of GABAergic signal transduction on suppressive effect of sevoflurane on *Per2* expression. Sevoflurane was applied to SCN slice cultures from Per2-dLuc transgenic rats, and luciferase bioluminescence was monitored using a microscope equipped with a CCD camera. To investigate a detailed spatial property of sevoflurane effect, acquired time lapse images of the SCN were divided into small regions of interest (ROIs). The bioluminescence in the most of ROIs showed a clear circadian pattern, and the bioluminescence was repressed by sevoflurane application. We also examined the possibility that sevoflurane suppresses *Per2* expression through the modulation of GABA receptor activities. To investigate the role of GABA receptors in suppression of *Per2* expression by sevoflurane, we applied GABA receptor blockers to the SCN cultures. The suppressive effect of sevoflurane was totally diminished in the presence of GABA receptor blockers. These results suggest that GABAergic mechanism is indispensable for sevoflurane to suppress *Per2* expression in the SCN, indicating that sevoflurane may act via GABA receptor systems in the SCN.

(COI: No)

P3-298

Close relationship between histamine H1 receptor-expressing neurons and CRH neurons in the mouse hypothalamic paraventricular nucleus

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Paraventricular nucleus of the hypothalamus (PVH) is a satiety center that inhibits feeding. Several types of neurons have been found in the PVH that regulate feeding, including CRH, oxytocin (OXT), TRH, and Nesfatin-1 neurons. In addition to these types of neurons, we showed that histamine H1 receptor (H1R)-expressing neurons are also involved in feeding regulation (J Physiol Sci. 62:S209, 2012). In this study, we tested whether H1R neurons had something to do with these types of neurons. First, we examined whether H1R neurons coexpressed CRH, OXT, vasopressin (AVP) or TRH in the mouse PVH by use of double *in situ* hybridization (ISH) method. Secondly, we examined the effect of the ablation of H1R neurons (ibid.) on other types of neurons in the PVH. The ISH study showed that about half of the H1R neurons expressed CRH, but less than ten percent of them expressed OXT and/or AVP, and no H1R neurons expressed TRH. Secondly, the ablation of H1R neurons greatly decreased the number of CRH neurons, but had little or no effect on the number of OXT, AVP and TRH neurons. These results suggest that the PVH neurons can be classified into two groups, H1R/CRH group and OXT/AVP group. Although OXT neurons have been reported to regulate feeding, the present study indicates that H1R an/or CRH neurons also regulate feeding by a mechanism distinct from that of OXT neurons.

(COI: No)

P3-299

Dopamine release in the nucleus accumbens of estrous female rats during exposure to male odors

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In seminatural conditions, estrous female rats actively pace the timing of coital stimulation from male rats. During such copulation under female preferred pace, female rats show increased extracellular concentration of dopamine (DA) in the nucleus accumbens (NAcc), implying the anticipation of rewarding sexual stimuli. It has been known that estrous females show preference for odors from males over estrous females irrespective of presence or absence of prior sexual experience. A previous study in our laboratory, however, demonstrated that Fos expression in the NAcc core was increased after exposure to soiled bedding from male rats only in sexually experienced estrous females (Hosokawa and Chiba, 2007). These results may suggest that odors from males are not intrinsically rewarding for female rats despite the existence of male-directed odor preference in sexually naïve females. In the present study, we examined the change of NAcc extracellular DA concentrations during exposure to male odors in both sexually experienced and sexually naïve estrous female rats using in vivo microdialysis combined with HPLC. DA concentration in the NAcc significantly increased after exposure to male odors, not only in sexually experienced but also in sexually naïve estrous females. However, the magnitude of the increase in DA during exposure to male odors was greater in sexually experienced females than that in sexually naïve ones. These date suggest that male odors are intrinsically rewarding for females and that sexual experience contributes to increase the value of the reward.

(COI: No)

P3-300

Administration of NMDA antagonist shifts the interval timing peak rightward in rats

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Timing and time perception are fundamental to survival and goal approaching in all animals. It is known that animals have some special timing ability of intervals. However, neural mechanisms of time perception are still unknown. The purpose of this study is to investigate the effects of NMDA antagonist on timing behavior. Firstly, using six male rats of Wistar strain, approximately 3 month-old at the beginning of the experiment, we examined psychological expectation of the interval timing in laboratory experimental settings with the peak-interval (PI) procedure. Interval-timing refers to time estimation in the second-to-minutes range. In the PI procedure, rats were trained on a fixed interval schedule to press lever for food after a specified interval (30 seconds in this experiment) as signaled by a certain stimulus. The rats received reinforcement only for desirable response. Though with some individual variations, the distribution of the lever press responses eventually showed an apparent peak in the vicinity of 30 seconds. Secondly, after 30 sessions of trainings, NMDA antagonist was administered directly into the septum region of the brain via microinjection. As a result, the peak time shifted rightward and lever press responses increased. This result of this study suggests that the comparison between the rats administered with NMDA antagonist, NMDA agonist, dopamine agonist and antagonist may clarify neural mechanisms of the interval timing.

(COI: No)

P3-301

Differential effects of propofol and etomidate on hypnotic electroencephalogram stage and sleep-wake cycle in mice

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General anesthesia is the clinical state comprising multiple components such as sedation, hypnosis and immobility. $GABA_A$-R is the major target of general anesthetics which mainly responsible for sedation and hypnosis. Propofol and etomidate exert anesthetic actions via the same $GABA_A$-R $\beta 3$ subunit. Thus, we examined whether propofol and etomidate possess the similar of hypnotic action and the same effects on sleep-wake cycle or not, by analyzing EEG and EMG. We found that both anesthetics induced significant increases in theta EEG power after anesthetics administration. Interestingly, the occurrence rate of slow wave burst of propofol is significantly lower than that of etomidate. As for sleep-wake cycle, propofol extended the duration of no-rapid eye movement sleep compared with etomidate. We discuss the differential effects of propofol and etomidate on hypnotic-EEG-stage and sleep-wake cycle.

(COI: No)

P3-302

Establishment of an in vitro experimental system using a cell line to investigate the mechanisms of anesthesia

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The molecular mechanisms of the general anesthesia have still remained to be clearly elucidated. The inhalational anesthetic, sevoflurane, is the most used general anesthetics in human. Our recent studies revealed the following evidence: 1) Administration of sevoflurane reversibly suppressed expression of the clock gene Per2 in the suprachiasmatic nucleus (SCN). 2) The suppression of Per2 expression in the SCN was mediated via histone deacetylation in the Per2 promoter. 3) Sevoflurane altered the phase of Per2 expression rhythm in the SCN slice culture. 4) Inhibition of GABA receptors blocked the sevoflurane-induced phase shift of Per2 expression in the slice culture. To further investigate the molecular mechanisms and target sites of the general anesthesia, it was required to develop an in vitro experimental system. We developed a GT1-7 cell line stably expressing the luciferase gene under control of the mouse Per2 promoter (GT1-7:6D3) and compared the response to sevoflurane with that of the other stable cell line (RS182). GT1-7:6D3 cells showed luciferase activity in a circadian manner as well as RS182 cells. GT1-7:6D3 cells responded to sevoflurane and showed a decrease in the activity leading to phase delay. Whereas treatment of RS182 cells with sevoflurane induced no change in the phase of luciferase expression. Now, we are examining the epigenetic events under anesthetic treatment.

(COI: No)

P3-303

A variant right hepatic artery with caterpillar hump formation: A case report with surgical implications

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Pub Med search reveals the presence of a few reports regarding the caterpillar right hepatic artery (CPRHA) (right hepatic artery with caterpillar hump configuration) in spite of its clinical relevance to laparoscopic cholecystectomy. During the dissection of abdominal cavity in 27 cadavers, we detected a case of trifurcated common hepatic artery into gastroduodenal artery, left hepatic artery and CPRHA in old man. After forming its characteristic caterpillar hump while passing ventral to the terminal part of common hepatic duct, the CPRHA passed through Calot'triangle giving its large cystic branch, then left the triangle deep to the cystic duct and gall bladder neck before its termination into the cystic plate. This case of CPRHA was associated with accessory left gastric artery stemming from left hepatic artery. The latter artery also gave rise to the right gastric artery. The accessory left gastric artery terminated at the gastroesophageal junction with terminal esophageal and fundic branches. The branching pattern of celiac artery was not typical; the splenic and common hepatic arteries arose from a common hepato-splenic trunk while the left gastric artery originated separately at more proximal level. The clinical relevance of the present case report to hepatobiliary surgery will be discussed.

(COI: No)

P3-304

The Observational Study of the Knee Articular Cartilage among Cadavars in S University

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We performed the observational study of the cadavars for medical training from 2012 to 2014 in S university, faculty of medicine as subjects. Our aim is to grasp the condition of the knee joint cartilage in the general elderly. The 81 cadavers, with the approval of the bereaved, were observed. The years of age were 85.15 ± 9.92 years (minimum 61 year-old, Max 103 year-old). The subjects were 38 males, and 43 females. The male cadavars were statistically younger than female cadavars by Welch t-test ($p<0.0001$) (80.47 ± 8.94 years in male vs 89.28 ± 8.94 years in female). To observe the knee joint cartilage, the femoral articular surfaces were divided 5 areas, the tibial articular surfaces were divided 3 areas, and the patella articular surfaces were divided 2 areas. We assessed the articular cartilage by each area, as following four steps, which were "1"= normal, "2"= fibrosis, "3"= ulcer-like, "4"= missing. Total score is up to 40 points, and a minimum 10 points by each knee. We also recorded for the presence of osteophytes of each area. Though the score in the right knee has positively related with those in the left knee (peason $r = 0.83$), the scores in the right knee were significantly higher than those in the left knee by paired t-test ($p = 0.015$). Comparing the total scores between in the presence and in the absence of femur osteophytes, the scores in the presence group are higher than those in the absence group by Welch t-test ($p<0.0001$).

(COI: No)

P3-305

Observation of the bipennate portio anterior of the soleus muscle using ultrasonic image

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The bipennate portio anterior of the soleus muscle is unique to the bipedal walking humans. We examined donated cadavers to investigate the types of the positional correlation between the calcaneal tendon and the sagittal tendon of the bipennate portio anterior. The purpose of this study is to determine whether or not it is possible to observe the deepest layers of the soleus muscle using ultrasonic image on donated cadavers and comparing those images with visual observations during a dissection. Two cadavers fixed with 10% formalin solution were observed in this study. After conducting sonograms on the four lower extremities to confirm the sagittal tendon, dissections were performed and visual observations were compared with the image-based observations of the sagittal tendon. Ultrasonic images of the posterior surface of the legs of five adult males and females were also used to compare the length and direction of travel of the sagittal tendon. It was suggested that when the sagittal tendon was present, the direction of travel of the band can be confirmed using ultrasonic images. However, using ultrasonic images, it is difficult to identify where the sagittal tendon begins and the point of transition to the Achilles tendon. It was also suggested that there was a tendency for the tendon to be identified as shorter than was actually apparent during visual observation.

(COI: No)

P3-306

Morphological analysis of the trapezius: muscle fibers in the three divisions

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The trapezius is diverse in shape and molds the outline of shoulder by keeping scapula in position. The 15 trapezius muscles specimens were photographed before and after removal of the fascia. The shape of trapezius was quite diverse at first, but became similar after removal of the fascia. The trapezius was divided into three parts depending on the insertion on the clavicle, on the acromion and scapular spine, and via distal tendon on the medial part of the spine. The proximal tendon was developed in the superior and middle part. In the superior part, the tendon protruded more on the superficial side, whereas in the middle part it protruded widely on the deep side to provide wide attachment for the muscle. In the superior part, the muscle fibers in the higher location were more inclined and longer and those in the lower location were nearly horizontal and shorter. The relation was reversed in the lower part. In the middle part the muscle fibers were almost horizontal and homogeneous in length, since the shape of the protrusion of proximal tendon and of skeletal insertion were fit well. The superior and inferior parts were slender sheet and the middle part was thick and voluminous. The middle part was most well developed and functioned as main sustainer of the scapula, whereas the superior part was poor and ancillary, and the inferior part function as abductor of the scapula.

(COI: No)

P3-307

Functional anatomy of the acromioclavicular ligament based on its macroscopic fiber analyses

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Introduction: The acromioclavicular (AC) ligament connects between the acromion and the lateral clavicle. The ligament has been illustrated as running nearly vertical to the AC joint surface, and has been described to interlace with the aponeuroses of the trapezius and deltoid muscles. While there are a lot of researches regarding measuring dimensions of the AC ligament for a distal clavicle resection technique, detailed macroscopic researches have not been done. Our objective for this study was to investigate the morphology of the ligament in detail.
Methods: We used 20 shoulder girdles of 11 cadavers in the anatomical practice at Tokyo Medical and Dental University. After extracting the scapula and the lateral half of the clavicle en bloc, we observed the ligament macroscopically.
Results: The AC ligament could be divided into two parts, posterior and anterior bundles. The well-developed posterior bundle ran obliquely from the anterosuperior part of the acromion to the posterior part of the lateral clavicle. In contrast, the anterior bundle was poorly developed and connected between the anterior surface of the acromion and the clavicle. The ligament was clearly separated from the muscles.
Discussion: Orientation of the posterior bundle passed obliquely over the joint surface, which might act as a constraint against the posterior translation of the clavicle in relation to the acromion.
(COI: No)

P3-308

Morphometric study of molar root furcation area and its relation to periodontal tissue destruction in Japanese populations

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Furcation involvement in periodontal disease has long been a challenge for dentists. As the destruction of the periodontium progresses apically, the furcation of multirooted teeth is exposed, leading to irreversible bone loss. Therefore, a thorough understanding of root anatomy is essential for proper diagnosis. However, little is known about three-dimensional (3D) morphology of molar root furcation (MRF) area and its relation to periodontal disease. The aim of this study was to establish a 3D measurement technique of MRF in 20 mandibular molars. In addition, 19 extracted molars of Japanese patients were investigated to evaluate their relation to periodontal parameters, including probing pocket depth, attachment level and bleeding on probing. Virtual images were generated from micro-CT imagery to quantify the MRF area. Variables such as root trunk length, furcation entrance, root separation and cervical enamel projection were evaluated. Our result showed similar values for the variables measured by conventional two-dimensional methods. In conclusion, 3D measurements of MRF area were successfully established. Additional data are still needed to assess relationship between 3D morphology of MRF and periodontal tissue destruction.
(COI: No)

P3-309

Anatomical variations of the lingual artery: a case report

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Recently crucial bleeding is the focus of preventive care in dental implant surgery. On the anatomical survey of blood vessels in oral floor, we newly found bilateral variations in arising pattern of the lingual artery in a case. On the right side, the deep lingual artery and the sublingual artery divided from common trunk which ran forward lateral to the hyoglossus, and this trunk arose from the facial artery just proximally to the position at which the submental artery arose. At the external carotid artery level, the original but remnant lingual artery arose just below the facial artery to distribute only the tongue root covered by the hyoglossus, entering to this area just above the greater horn of the hyoid bone. On the left side, the sublingual artery arose from the facial artery just proximally to the position at which the submental artery arose. The deep lingual artery, on the other hand, was the continuation of the lingual artery which arose from the external carotid artery far below the facial artery, making anastomosis with the sublingual artery in the sublingual region. The lingual artery of this side ran laterally to the hyoglossus during coursing the posterior half of this muscle, then penetrated the muscle and ran medially to the hyoglossus up to the anterior border of this muscle to continue to the deep lingual artery. In this side, there seemed to be no remnant lingual artery seen in opposite side. Providing the detailed portrait of arterial variations may be of clinical importance in dental implant surgery and preoperative radiologic examinations.
(COI: No)

P3-310

A morphological study of the maxillary anterior wall in Japanese population

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Maxillary bone between a nasal cavity and the maxillary sinus is available for the dental implant to bury. Since the bone around the maxillary sinus is thin, the dentist should be care for the dental implant placement. We observed the maxillary bone thickness in the cross-section of the alveolar process of the maxillary bone.
The head of the 23 cadavers (male 12 bodies 20 side 101-52 years old, female six bodies 11 side 100-71 years old) for of the dissection training at the Nihon University School of Dentistry at Matsudo in 2013. We cut the horizontal section on the upper part of 1cm from the alveolar crest at the maxillary canine part by the belt saw, and in addition 1cm upper part of the section was cut off. The cross-sections were photographed with a digital camera and drew a circle on the bone of the point of intersection of the canine fossa, the maxillary sinus and the nasal cavity with image analysis software and calculated the diameter of the circle.
The size of the circle did not have the significant sex differences, the significant difference by having tooth or not. As for the average of the diameter of the circle, in the 1cm upper part, in 6.1mm, the 2cm upper part, it was 4.7mm from the alveolar crest. The standard of the diameter of an implant is 3.75 mm and the frequency of the size not more than it is 9 sides in 1 cm and 13 sides in 2cm. In these areas, the dental implant may perforate the maxillary sinus, the nasal cavity and the canine fossa, and therefore attention is necessary.
(COI: No)

P3-311

Morphological analysis of the vastus lateralis and intermedius of the quadriceps femoris

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Anatomy of the vastus lateralis (VL) is complicated. VL is clinically divided into the long and oblique heads (VLL, VLO) and in addition its border toward the vastus intermedius (VI) is frequently blurred because of partial fusion origin's form these muscle. Eighteen specimens of the quadriceps femoris were detached from the skeleton of cadavers with preserving the periosteum and intermuscular septum. The field of origin of VL and VI was demarcated on the deep surface of specimens. The insertion of VLL and VLO was also analyzed as regards to the insertion tendon.
The origins of these muscles were classified into 3 types. In type I, the three origins were continuous (9/18). In type II, the origin of VI was separated from the origins of VLO and VLL (8/18). In type III, the origin of VLL was separated from the origins of VLO and VI (1/18). The insertion of VLL was classified into 3 types; type A with wide insertion tendon on the deep surface (10 cases), type B with intermediate insertion tendon (2 cases) and type C with narrow insertion tendon at the lateral and medial border of the muscle (6 cases). VLO inserted on the tendon of VLL either without additional insertion (type X, 7 cases) or with insertion tendon onto the patella (type Y, 11 cases). It was revealed that VL and VI made a single muscular body in most of the cases, and the separation of this muscular body occurred in various places. This fact indicated that the quadriceps femoris had three heads instead of four.
(COI: No)

P3-312

Comparison of vapor levels of formaldehyde from embalmed human cadavers between males and females

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Formaldehyde (FA) is soluble compound, and used to embalm human cadavers for gross anatomy laboratory. It has been documented that FA vaporize from embalmed cadavers in laboratory. However little is known about evaporation level of FA in each cadaver and dissecting process. FA vapor levels have been compared among non-dissected (ND), skin-incised (SI), subcutaneous fat-removed (FR) and thoracoabdominal cavity-opened (TO) cadavers in our previous studies, and our results have shown increased FA levels in SI, FR and TO cadavers compared to that of ND. In this study, we evaluated the FA levels between male and female cadavers. FA was collected by active sampling method and evaluated by high performance liquid chromatography. Our data showed that the FA level increased in SI, FR and TO cadavers compared to that of ND in both of male and female cadavers. In particular, such increase was significant in SI and FR cadavers in males. In addition, we found that the FA levels are higher in female cadavers than in male cadavers. This sexual difference was significant in FR and TO cadavers. These data provide new knowledge about difference in vapor levels of FA from embalmed cadavers.
(COI: No)

P3-313

Right external iliac venous ring lacking the right common iliac vein

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Preaortic iliac venous confluence, also known as marsupial vena cava, is a rare congenital anomaly in which inferior vena cava or left common iliac vein is located anterior to aortic bifurcation or right common iliac artery. A very rare case of preaortic iliac venous confluence was found in a 84-year-old female cadaver. In this case, the confluence originated from right external iliac vein and drained directly into inferior vena cava. This confluence and right external iliac vein surrounded right common iliac artery to form "external iliac venous ring". In addition, right internal iliac vein drained into left common iliac vein and right obuturator vein draining into right external iliac vein. Embryogically, external iliac venous ring in our case may represent a form that developmental preaortic and postaortic iliac veins both persist. The external iliac venous ring has clinical importance especially during central venous cauterization from right external iliac vein. The other venous variations are no less clinically important in retroperitoneal surgery respectively.

(COI: No)

P3-314

Anomalous course of the external carotid artery

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After the external carotid artery begins at the bifurcation of the common carotid artery, at the level of the upper border of the thyroid cartilage, it ascends usually medial to the stylohyoid muscle. It reaches to the region between the neck of the mandible and the mastoid process, where it ends by dividing into the superficial temporal and maxillary arteries. However, it is known that this artery sometimes runs between the posterior belly of the digastric and stylohyoid muscles, rarely lateral to the digastric muscle. So the incidence and the branching patterns of such anomalous external carotid artery were investigated in a total of 550 bodies or 1100 head sides of Japanese subjects, donated for student dissection at Kumamoto University from 1994 to 2014. With the exception of 3 head sides in which the course of the external carotid artery was not clear, the external carotid artery running between the digastric and stylohyoid muscles were found in 42 (3.8%) out of 1097 head sides. Further, in 23 out of them, the external carotid artery ran between the stylohyoid branch of the facial nerve and stylohyoid muscle. In the remaining 19 head sides, the stylohyoid branch has been cut and there was no instance in which the stylohyoid branch of the facial nerve ran obviously medial to the external carotid artery. The external carotid artery running lateral to the digastric muscle were found in 4 (0.4%). In this research, I examined which branch has a possibility of being a vestige of such anomalous external carotid artery and discussed its process of formation.

(COI: No)

P3-315

The morphologic study on a course of the maxillary and the posterior deep temporal arteries

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Objectives: The distribution of the maxillary artery (Mx) is important landmarks for surgery. The course of Mx was classified into lateral type and medial type. The lateral type runs superficially to the lateral pterygoid muscle (LP), and the medial type runs deeply to the LP. The order of divergence of the Mx in the lateral type was the middle meningeal artery(MM), the inferior alveolar artery (IA), the posterior deep temporal artery (PDT). On the other hand, the order of divergence of the medial type was common trunk of the MM and IA, the PDT.
Material and Methods: We studied the course and order divergence of the Mx at the dissection training in 2013. We compared the data which was obtained at the Nippon Dental University with the present study. We injected resin to the external carotid artery (EC) after dissection. After the injection, we confirmed previous EC distribution.
Results and Discussion: The course of the Mx in the lateral type was 90%, in the medial type was 10%. Double maxillary arteries were observed in the most deeper medial type of the Mx. It was very rare case. The PDT which deeply run to the LP was found. A study of arteries in the maxillofacial region is clinically very important. We believe the study of blood vessels will be useful for many clinical fields.

(COI: No)

P3-316

The relationship between age-related changes in the lumbar spines and sacroiliac joints

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Back ground: The purpose of this study was to clarify the relationship in the age-related change of lumbar spine (LS), these of sacroiliac joints (SIJ) and other major joints, and the shape of the SIJ surface.
Method: SIJ and LS (osteophyte of vertebral body and degenerative changes of zygapophysial joint) and periarticular osteophytes of six major joints in 42 modern male skeletons were quantitatively examined macroscopically. A corrected index of age-related change, which was the gap value (Gap), was calculated. Moreover, the degree of curvature in the posterior border line around the iliac auricular surface was calculated as a quantitative indicator, constriction ratio (CR). Some correlation coefficients were examined with Gaps and CR.
Results: Certain degree of positive relationship between Gap in the major six joints and those in SIJ was indicated. Furthermore, there was a certain positive correlation between left SIJ and LS. There was a tendency of negative correlation between Gap in the vertebral body and the mean value of CR of both sides.
Conclusion: It was suggested that these results were relevant to the age-related changes of SIJ and LS. The relationship between the degree of osteophytes in the vertebral body and shape of the auricular surfaces indicated that the difference in stability of SIJ might affect the extent of the mechanical stresses occurring in the lumbar vertebral bodies.

(COI: No)

P3-317

Muscle Architecture of the Triceps Surae Muscle

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The major ankle plantar flexor is the triceps surae muscle (TSM), which comprises the medial gastrocnemius (MG), lateral gastrocnemius (LG) and soleus (SOL) muscles. The MG and LG cross both the knee and ankle joints, whereas the SOL is a plantar flexor that crosses only the ankle joint. The SOL has a unique architectural feature characterized by a pinnated portion on its ventral surface. The present study was performed to quantitatively evaluate the muscle structure and compare the composition of muscle fiber types of the TSM.
The gross anatomical features of 138 formalin-fixed cadavers (83 males and 55 females) at our laboratory were studied from 2009 to 2014. The MG, LG and SOL were observed from both the dorsal and ventral sides, and each part of the muscles and all tendons were measured. Additionally, the histological features of the TSM were observed in five human cadavers. The muscle fibers were categorized into slow- and fast-twitch fibers by immunohistochemical staining.
The LG was a bipennate muscle that contained an intramuscular tendon, while the MG was a unipennate muscle with no intramuscular tendon. Moreover, a bipenniform muscle structure was mostly present in the deep ventral region of the SOL. The mobile end of this part of the tendon of the SOL is called the sagittales sehnenblatt. The minimum width of the Achilles tendon was positively correlated with the maximum width of the belly of the TSM. The percentage of slow-twitch fibers was higher than that of fast-twitch fibers in the MG, LG and SOL.

(COI: No)

P3-318

Ethics Subcommittee on the Cadaver Study in Kyorin University

Matsumura, George (*Dept. Anat., Sch. Med., Kyorin Univ., Tokyo, Japan*)

Presently, in contributing the findings of cadaver study to most medical journals, the deliberation and approval by the Medical Ethics Committee is an essential requirement. In 2012, to carry out surgical training and research using donated cadavers lawfully, "the guideline of the autopsy in education and research of clinical medicine" was presented by Japan Surgical Society and the Japanese Association of Anatomists. The guidance specifies the Ethics Committee of each medical school should recognize implementation of the cadaver study, after examining the legitimacy of the research procedure sufficiently. However, most of the Ethics Committee were organized for deliberating whether the patients' human rights, dignity, and their personal information are protected appropriately in clinical and/or hospital studies. Conventionally, cadaver studies were performed according to "Autopsy Conservation Act", and not regarded as deliberation matters by the "Clinical" Ethics Committee. For this reason, many cadaver studies were not able to undergo deliberation of the Ethics Committee. In Kyorin university, the Ethics Subcommittee of the Cadaver Study was organized to examine the ethicality of research using cadavers. The requirements for undergoing examination are reported.

(COI: No)

P3-319

The great cardiac vein and the anterior interventricular branch of left coronary artery covered with myocardium: A case report

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Myocardial bridges (MBs) are one of the anatomical variations of the coronary artery that is covered by myocardium (Myo). We previously reported that the cardiac veins and autonomic nerves located on the surface of the Myo in the case of the MB. In the present study, we show a rare case that the Myo enveloped not only the anterior interventricular branch (AIB) but also the great cardiac vein (GCV) in 75-year-old male Japanese cadaver at the anatomical dissection in the Nippon Dental Univ. Niigata. The proximal part of the AIB and GCV was covered with Myo. The Myo in this part covering the GCV was thinner than that of the AIB. The fine vein penetrated the superficial layer of this overlying Myo along this muscle bundle. In the middle part, the AIB ran deep to the Myo but the GCV passed the external surface of the Myo. In the distal part, the AIB and GCV lied on the Myo. A branch of the autonomic nerves entered Myo along with the AIB, and the remaining nerves ran over the surface of the Myo. Both nerves passed along the distal AIB. Coronary vascular precursor cells derive from the epicardium in the early stage of development (reviewed by Brade et al., 2013). Hypoxia inducible factor1 α (HIF1 α) regulates migration of these cells into the Myo (Tao et al., 2013). Taken together with the previous data, the present case suggests that HIF1 α may affect not only the coronary artery but also the cardiac vein.

(COI: No)

P3-320

Morphological study of the lateral occipital nerve using a technique of the nerve fiber analysis

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Current anatomy textbooks describe that the greater occipital nerve (C2:posterior ramus), the minor occipital nerve (C2:anterior ramus), the third occipital nerve, and the great auricular nerve (C3:anterior ramus) are distributed from medial to lateral side in the occipital region, and the third occipital nerve is distribute in lower region to the greater occipital nerve. Especially, the cutaneous branch of C1 is not generally distributed in the region. However, the lateral occipital nerve (including C1/C2 cutaneous branch) is rarely distributed in the region between the greater and lesser occipital nerves. The lateral occipital nerve was recognized as the lateral branch of the posterior ramus of the C1/C2, and ran upward outside of the occipital artery. The lateral occipital nerves at right and left sides were observed in macroscopic anatomy seminar at Niigata in 2013. This nerve had a similar feature as we mentioned before. On the other hand these nerves were characterized that was not branched from the lateral branch of the posterior ramus of the C1. However, the nerve originated from the anterior ramus of the C1. To sum up our study, we confirmed the root of the lateral occipital nerve, by using the technique of nerve fiber analysis.

(COI: No)

P3-321

Variations in the vessels connecting the posterior tributary of the left renal vein to the left ascending lumbar vein without communicating with other renal veins in a Japanese cadaver

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A rare variation was found in one of the two left renal veins in a 94-year-old male cadaver undergoing routine dissection. The characteristic findings in the cadaver included, in addition to the primary left renal vein, the presence of a posterior left renal vein draining to the left ascending lumbar vein without communicating with the inferior vena cava and other renal veins. Variations in the number and arrangement of the vessels terminating in the renal veins are common, but to our knowledge, variation similar to our findings has not been previously reported. This variation may represent an immature form of the complicated development of the renal vessels.

(COI: No)

P3-322

An anatomic variation of the coracoacromial ligament: a case report

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We observed a variation of the coracoacromial ligament of the right shoulder in an 82-year-old Japanese female cadaver during dissection at Tokyo Medical and Dental University.

The variant ligament attached to the base of the coracoid process, which was located lateral to the insertion of the superior transverse scapular ligament. It was directed posterolaterally and attached to the anterior border of the acromion. The ligament was 7 mm in width and 32 mm in length. The thickness was 5 mm at the coracoid process and 2 mm at the acromion. The ligament was covered by the clavicle and the trapezoid part of the coracoclavicular ligament. The anterior border was continuous with the proper coracoacromial ligament. The supraspinatus muscle was under the variant ligament.

The variant ligament in this report is similar to the third part of the coracoacromial ligament which was reported by Pieper et al. (1997). However, the attachment at the coracoid process is next to the insertion of the transverse scapular ligament or the coracoclavicular ligament. We consider that the variant ligament might have as close relations with these ligaments as the proper coracoacromial ligament.

(COI: No)

P3-323

Anatomical study on the flexor digitorum superficialis in common marmoset (*Callithrix jacchus*)

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The common marmoset is a New World monkey and uses arboreal locomotion. They can hang vertically from trees and leap between them. Therefore, digital flexors of the forelimb of this species, especially the flexor digitorum superficialis (FDS), are important to perform this style of arboreal locomotion. We dissected the upperlimbs of the adult common mamoset (two cases) and found that the marmoset FDS originated from the medial epicondyle of the humerus as a common origin with the pronator teres, the flexor carpi radialis, and the palmaris longus. The muscle belly for the 5th digit was independent from the other parts of the FDS, and it represented a two-bellied muscle. In one case out of two, the proximal belly of this two-bellied muscle received a twig from the ulnar nerve. The muscle bellies for the 2nd, 3rd, and 4th digits were highly fused each other. The previous study of the human FDS (Ohtani, 1979) showed that the FDS was divided into the superficial layer for the 3rd and 4th digits and the deep one for the 2nd and the 5th digits. The deep layer of the human FDS has the intermediate tendon from which two distal bellies originate and give rise to the tendons for the 2nd and 5th digits. The FDS of the marmoset for the 2nd digit has more fleshy part in comparison with that of human FDS, implying that the marmoset FDS is able to make their digits flex more strongly, which is suitable for climbing on trees.

(COI: No)

P3-324

"The medial cutaneous branches" of the upper cervical dorsal rami do not originate from the medial branches of the dorsal rami of the cervical spinal nerves

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The dorsal cutaneous branches in the upper cervical and the occipital regions have been believed as the homologous nerves with the medial cutaneous branches of the dorsal rami of the upper thoracic nerves, however, the true medial branches of the dorsal rami of the cervical nerves to the transversospinal muscles existed independently of the cutaneous branches. These cutaneous branches were accompanied with the branches to the semispinalis capitis that did not only receive those branches but also the branches from the lateral branches widely with the communications between them. Thus the former were more familiar to the lateral branches than the medial branches. We had reported the details of the courses and the distributions of the lower thoracic lateral branches of the dorsal rami. When the branch to the longissimus muscle takes the most medial course penetrating the intertransverse ligament, it looked like as medial branch. Therefore, we called those branches as "the intermediate branches". The branches to the semispinalis capitis with the cutaneous branches are resembled to the intermediate branches in the lower thoracic region. Therefore, we advocate that the upper cervical dorsal cutaneous branches should be called as the intermediate cutaneous branches, and the semispinalis capitis also should be considered as the medial part of the longissimus capitis.

(COI: No)

P3-325

Re-considering the pathogenesis of Achilles tendonitis based on tendinous-fiber analysis findings

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To re-examine the conventional pathogenesis of based on the structural characteristics, we studied detailed anatomical reviews of the Achilles tendon (AT) by analyzing the fiber bundles that comprise it, and by examining the relationship with the running of the plantaris tendon (PT). We examined 90 lower extremities (47 cadavers). In all cases, we observed inward twists of the comprising fiber bundles beyond the narrow part of the AT. Specifically, we observed regular twists between the fiber bundles originating from the medial gastrocnemius muscle and those from the soleus muscle. We also found that posterior to the tendon, part of the subcalcaneal bursa (synovial fold) fitted between the fiber bundles, which were separable. The most common arrangement of the plantaris tendon was Anson's type III. Around the calcaneus, the AT and PT were covered by a common fascia. These results suggested that fiber bundles may exist when internal rotation occurs in the internal parts of AT. As three functional fiber-bundle areas exist between the fiber bundles in the distal part of AT, the pathogenesis so-called a "saw-like action" may have anatomical basis. In addition, the existence of synovial folds that fit into the interior of the tendon insertion site is considered to be an important finding, because it is closely related to the synovial fringe for causing enthesitis.

(COI: No)

P3-326

A case study of both sides of the vertebral arteries passing through the 3rd transverse foramen and branches from the sympathetic trunk

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Right side of this case, the vertebral artery originated from the bifurcation point of the common carotid and subclavian arteries. Left side of this artery originated from the aortic arch as the 3rd branch from the arch. Both sides of these arteries ascended anterior of the transverse process, turned backward between the longus colli muscles to pass through the 3rd to 1st transverse foramen. These arteries branched off meningeal branches and passed through the dura mater at C1 vertebra. In addition, both sides of the subclavian arteries branched off additional vertebral arteries passed through the 6th and 5th transverse foramen. The right additional artery distributed the anterior surface of the dura mater of the ventral root for the C5 spinal nerve. The left one divided into medial and lateral branches. The medial branch passed through the dura mater from the anterior surface of the C5 ventral root, distributed the anterior surface of the spinal cord. The lateral branch ascended within the 4th transverse foramen and branched off some twigs to the periosteum. Branches from the sympathetic trunk and ganglion passed among the longus colli muscles segmentally and ran along the upper entering vertebral arteries and additional ones. These pathways of the sympathetic branches were thought to be the route of the intersegmental artery during the developmental period.

(COI: No)

P3-327

A case study of the thymic artery which passed deeply to the left brachiocephalic vein

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The major part of the thymus is located in the superior mediastinum of the thorax and lies superficially to the left brachiocephalic vein. Arterial supplies of the thymus are derived from branches of the internal thoracic and inferior thyroid arteries. It reflects that the thymus descend superficially to the left brachiocephalic vein. However, a case of the thymic artery which passed deeply to the left brachiocephalic vein was observed at the 8th Macroscopic anatomical seminar in Niigata. The arterial supplies to the right lobe of thymus were derived from the branches of the right common carotid and internal thoracic arteries. The arteries to the left lobe were supplied from branches of the left inferior thyroid, left internal thoracic and right common carotid arteries. The branch of the right common carotid artery passed deeply to the left brachiocephalic vein. Other arteries to the thymus passed superficially to the left brachiocephalic vein. Based on the relationship between the thymus and neighboring organs, it is more common the thymic artery runs superficially to the left brachiocephalic vein. The studies of locational relationship between the thymic artery and the left brachiocephalic vein could not found in any previous reports. We will report the rare case of the thymic artery.

(COI: No)

P3-328

What does the recurrent laryngeal nerve of dolphins curve around?

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Background: In mammals, the recurrent laryngeal nerve (RLN) curves around the ligamentum arteriosum (LA) on the right side and around the subclavian artery (SbA) on the left side. This asymmetric travel of the right and left RLN comes about because of different embryological development of each aortic arch.
Materials and Methods: We dissected a rough-toothed dolphin and a Pacific white-sided dolphin which stranded on the beaches of Japan.
Results: In both specimens, the brachiocephalic trunk (BT) gave off the common carotid artery, SbA and costocervical trunk (CT). The SbA was ventral to the subclavian vein and vagus nerve (VN). The right RLN arose from the VN where it crossed in front of the CT. It curved around the CT and then cranially on the right side of the trachea. On the left side, the RLN curved around the LA. The internal thoracic artery (ITA) gave off some thin ventral intercostal arteries and its thick terminal branch supplied the diaphragm.
Discussion: These results suggests that in dolphins the primary (dorsal) SbA disappears remaining the CT at a certain embryological stage and that the flipper receives a secondary (ventral) SbA which passes ventral to the VN like in turtle, crocodile and chick embryo.

(COI: No)

P3-329

Organization of the neck epaxial musculature of fetal pigs

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Organization of the neck epaxial musculature was investigated macroscopically in fetal pigs. In the most outer layer of the neck epaxial musculature, m. splenius originated from the median line at the all cervical and upper thoracic vertebrae. It splited to three parts, and inserted onto cranium and the transvers process of the atlas. It was innervated with dorsal rami of C2 to C5. In the middle layer the m. longissimus cervicis, atlantis and capitis existed. In the deep layer the m. biventer cervicis existed dorsomedially, and the m. complexus vetntrolaterally. The m. biventer cervicis originated from articular processes of upper thoracic vertebrae and inserted onto cranium just laterally to median line. It was innervated with dorsal rami of C2 to C6. The m. complexus originated from transverse processes of cervical vertebrae and inserted onto cranium laterally to the m. biventer cervicis. It was innervated with dorsal rami of C1 to C4, In the deepest layer the neck trasversospinalis system existed below 2nd cervical vertebrae and the mm. suboccipitales existed between 2nd cervical vertebrae and cranium. Innervation pattern was investigated in detail at every segment and organization pattern of the neck epaxial musculature will be discussed.

(COI: No)

P3-330

The relationships between the structure of digestive organ and ecotype of Cephalopoda

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Decapodiform cephalopods mainly eat crustaceans, fish and mollusks. In general, digestive organs morphologically be affected by the differences in food. However, morphological differences in digestive organs were shown among cephalopods. Then, it is considerable that lifestyles may affect the morphology of digestive organs. In this study, the relationships between the morphology of digestive organs and ecotypes of decapodiform cephalopods were surveyed.
Six species of adult decapodiform cephalopods representing five families and two different lifestyles were used. Pelagic species included *Todaredes pacificus* (n=4), *Loligo bleekeri* (n=4), *Loligo edulis* (n=4), and *Watasenia scintillans* (n=4), all of which have elongated bodies and swim actively. Benthic species included *Sepia lycidas* (n=4) and *Euprymna morsei* (n=4). Specimens were dissected and digestive organs were exenterated. The following digestive organs were examined: stomach, caecum, digestive gland and digestive duct's appendages. The percentage ratio of each organ's weight to total body weight was calculated by adjusting body mass for all six species.
Pelagic species possessed larger caecum and smaller stomach, digestive gland, and digestive duct's appendages. In contrast, benthic species had larger stomach, digestive gland and digestive duct's appendages, and smaller caecum. The speed of digestion is faster in the pelagic species to swim actively than in the benthic species. Pelagic species encounter more food than benthic species. Then, pelagic species may have smaller stomach for get less food and absorb nutrient faster in larger caecum. Benthic species may get more food in stomach and absorb slower in smaller caecum. And, the nutrient may also absorb in larger digestive duct' appendages and store in larger digestive gland in benthic species.

(COI: No)

P3-331

Dorsal derivative layer of the subcutaneous trunk muscle in the house shrew, *Suncus murinus*

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The house shrew, *Suncus murinus*, is a loose-skinned animal. Although this feature is probably due to the well-developed cutaneous muscles, the skin musculature in the family Soricidae has been described only briefly in earlier works. To understand the multilayered structure of the subcutaneous trunk muscle (STM) in the house shrew, especially focusing on the derivative layer in the dorsum, ten male specimens were dissected. The nomenclature followed that of Ura (1937), who stratified it into two layers: fundamental and derivative. The STM of the house shrew configurated a thin sheet and enclosed the entire body except for the limbs. The nerves supplying the STM were branches of the caudal pectoral nerve. The fundamental and derivative layers could be distinguished based on whether or not it kept the attachment on the humerus. The former, having humeral insertion, was composed of M. humeroabdominalis and M. humerodorsalis. The latter was lost humeral insertion and mainly spread superficial to the fundamental layer. The derivative layer was composed of M. ventralis superficialis, M. dorsalis superficialis, and M. dorsolateralis. M. dorsalis superficialis expanded well in the dorsum and acquired secondary attachment to the humerus and scapula. Only M. dorsolateralis was recognized deep to the supplying nerves. By the innervation and the order of stratification, it is inferred that the nuchal and axillary parts of M. dorsalis superficialis is finally differentiated from the derivative layer.

(COI: No)

P3-332

Relation of segmental variation in the lumbosacral plexus to length of the 12th rib in macaque specimens

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Segmental variation in the lumbosacral plexus has been studied in cadavers, and the evidence suggests that such variation is related to the length of the 12th rib;namely, a short 12th rib exhibits cranial deviation in the plexus and a long rib exhibits caudal deviation. We examined the relation between the length of the 12th rib and segmental composition of the lumbosacral plexus in 10 macaque specimens(20 sides). The furcal nerve(FN)-the boundary between the lumbar and sacral plexus-was used as an index of plexus arrangement. The length of the 12th rib was divided by the width of the proximal tibia-a common indicator of body size-to reduce bias arising from differences in the specimen body size, and the index is thus referred to as the"12th rib/tibia index". Segmental variation in the FN was roughly classified into 3 groups on the basis of whether the FN originated at L5(FN L5 group), L5 and L6(FN L5+L6 group), or L6(FN L6 group). The average 12th rib/tibia index was 2.3 for the FN L5 group, 2.42 for the FN L5+L6 group, and 2.66 for the FN L6 group. The length of the 12th rib was longer when the FN originated at a lower segment. These findings are relevant to the caudal extent of the thoracic region and suggest that variation and the present observed relation are common in primates. This work was supported by the Cooperation Research Program of the Primate Research Institute of Kyoto University.

(COI: No)

P3-333

Comparative anatomy of human posterior auricular muscle and cervicoauricularis muscle in mouse

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In human, the pinna resides just lateral to the head. On the other hand in quadrupedal mammals, the position of pinna usually extend posteo-supero-laterally to overhang neck and shoulder region. This morphological variation is accompanied with the structure of auricular muscle posteriorly attached to the pinna. Compared to the small size of posterior auricular muscle(PAM) in human, the same muscle develops in size in quadrupedal mammals and the origin goes down to the nuchal ligament which is named cervico-auricularis muscle(CAM). This muscle has been reported innervated solely by the posterior auricular nerve(PAN) of facial nerve (VII). In this study, we investigated the innervation of PAM and CAM using human cadaver in systematic anatomy (n=1) and Wistar rat (Rattus norvegicus) (n=1). In Wistar rat, the insertion (pinna) side of CAM is innervated by the PAN, while the origin side is innervated by the second dorsal branch of cervical nerve (DCN). Thus, we confirmed double innervation of CAM with nerves from head and neck, respectively. We affirmed that cutaneous muscle of trunk in the back of Wistar rat was innervated by the DCN in the same manner. These results raise the question whether CAM (PAM in human) is simply a posterior part of facial muscle. On the other hand, human PAM fits in the temporal region and solely innervated by PAN. We discuss the morphological variation of PAM and CAM in line with evolutionary change of head position in human.

(COI: No)

P3-334

Evaluation of PED procedure between surgery for patients and training using fresh cadavers

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Introduction: Although percutaneous endoscopic lumbar descectomy (PED) is the least invasive disc surgery procedure under local anesthesia, this procedure requires skill and experience. There is no doubt that the ideal learning PED procedure is in a real operating room (OR) but the standard surgical teaching of PED procedure in the OR is difficult for three reasons; local anesthesia, injury of lumbar nerve root and the need for advanced technique.
Purpose: The purpose of this study is evaluation of the differences between surgery for patients and training using fresh cadavers.
Methods: Three fresh cadavers underwent the PED procedure at the cadaver laboratory in Taiwan. The procedure included the use of 3 mm cannulas from the posterolateral approach, and removal of the nucleus pulposus with pituitary forceps after dyeing it using indigo methods.
Results: Fresh cadavers still have the same stiffness or viscosity as biological bodies. Discectomy of lumbar disc using fresh cadavers is the nearest simulation of the surgical procedures such as color of the epidural vessel, color of annulus fibers or lumbar nerve roots. Although shortening of the surgical learning curve should be obtained outside the OR, sufficient training using the plastic model or an animal could not be provided.
Conclusion: Training in the PED procedure using fresh cadaver may provide a useful way for surgeons to obtain skill.

(COI: No)

P3-335

Accessory mental nerve found during a gross anatomical dissection course

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Introduction: The incidence of the accessory mental foramen was reported as 2.0% to 11.9%. The accessory mental foramen is diagnosed easily by using three dimensional computed tomography (3D-CT). However, there are few reports of examining the distribution of accessory mental nerves (AMNs) branching off from the accessory mental foramina. In this study, we investigated the distribution patterns of the AMNs using Sihler's staining and transparency technique.
Materials and methods: In a gross anatomical dissection course in our medical school in 2012, we found a case which had right accessory mental foramen and AMN. We performed Sihler's staining technique for the mandibular bone and overlying skin, which makes the soft tissues transparent with staining the nerve deep blue.
Results: The right AMN mainly distributed to the right angular region and the right mental nerve mainly distributed to the right inferior labial and mental regions. Whereas, the left mental nerve distributed to the left angular, inferior labial, and mental regions.
Conclusion: This study revealed that the AMN was a branch to the angular region in this case. We have found some cadavers which have accessory mental foramina in the mandibles and are examining them. To predict the distribution patterns of the AMNs, we have to analyze these mandibles in detail. Then these results will be useful to avoid neurovascular complications during implant surgery, nerve block, and other oral surgery procedures.

(COI: No)

P3-336

Tibial attachments of the lateral meniscus and the anterior cruciate ligament

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Although the laxity of the lateral meniscus (LM) is occasionally observed in anterior cruciate ligament (ACL) deficient patients, the anatomic background of these cases have still remained unclear. To investigate the anatomy of LM, with special reference to the positional relationships to the anterior cruciate ligament (ACL). Twenty four knees from 12 Japanese cadavers (6 males and 6 females) were used in this study. All cadavers were fixed in 8% formalin and preserved in 30% ethanol. ACL were cut off at femoral insertion, and all other supporting tissues including posterior cruciate ligament, capsule and collateral ligament were separated at the attachment of tibia. After macroscopic investigations, six specimens were randomly chosen, and histologic examinations were performed. Outer fibers of the anterior horn of LM extended to ACL, and seemed to be intermingled with ACL fibers in macroscopic observations. However, after histological examinations, a border between LM and ACL were clearly shown. Inner fibers of the anterior horn of LM ran beneath the lateral intercondylar tuberculum, and attached to the lateral intercondylar eminence. Fibers of the posterior horn of LM were separated into the anterolateral crus and the posteromedial crus, and attached to the posterior aspects of the lateral and medial intercondylar eminences respectively. In conclusion, ACL is adjoined with the outer fibers of the anterior horn of LM, and the posterior horn of the LM is firmly attached to the posterior aspects of the intercondylar eminences of the tibia.

(COI: No)

P3-337

Using the petrous part of the temporal bone to estimate fetal age at death

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Little is understood about the age-related changes in the petrous part of the temporal bone in fetal life. The purposes of this study were to examine documented skeletal remains of Japanese fetuses, to measure the length of the petrous part, and to develop diagnostic standards for fetal age-at-death estimation that could be applied to poorly preserved skeletons. The results indicated that it is possible to use regression equations to estimate age at death directly from the length of the petrous part of the temporal bone. The application of the present method to a different population led to a fetal age-at-death estimation with an error of less than 1 month. We also used the Bayesian estimation, which yielded posterior probabilities of age, conditional on being of a particular length of the petrous part. The reference table of estimated gestational age may provide an easy-to-use indicator of the fetal age at death. In conclusion, measurement of the petrous part of the temporal bone may offer a new methodological basis for forensic and bioarchaeological diagnoses of fetuses.

(COI: No)

P3-338

Mutated FcεRI β chain (D234A) affects signal transduction of mast cell but does not affect protein structure and thermal stability of FcεRI β chain protein

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High affinity IgE Fc receptor (Fc ε RI) is expressed on mast cells as a tetrameric receptor composed of the IgE-binding α chain, four-fold membrane-spanning β chain, and disulfide-linked homodimer of the γ chains. Fc ε RI acts as a signal amplifier in mast cells. The β chain contains ITAM, a conserved feature of many antigen receptors that imparts signaling competence.

We revealed the biological functions mutated β chain (D234A) in mast cell activation upon Fc ε RI engagement and demonstrated that D234A severely impaired Fc ε RI-mediated cytokine production (IL-6), however, did not impair degranulation. On the other hands, we previously revealed that β chain ITAM with the replacement of tyrosine to phenylalanine (FFF) impaired degranulation, however, did not impair cytokine production.

In addition, we investigated the structure that is part of Fc ε RI β chain wild type (β-WT, aa:143-235) protein and β-D234A(aa:143-235) protein by circular dichroism spectroscopy (CD). The far-UV CD spectra of β-WT and β-D234A are of an α-helical structure and β-D234A does not have any loss or collapse of α-helical content. Near-UV CD spectroscopy showed that a conformational change has not occurred for β-D234A. The transition curve for the thermal denaturation of β-D234A obtained from ellipticity at 222 nm was almost the same as that of β-WT protein. Gibbs free energy change (ΔG) of β-WT is not different from that of β-D234A. Our results suggest that new signaling pathway through the D234 of the β chain may exist.

(COI: No)

P3-339

The striatal neuronal activity quantified by activation-inducedmanganese-enhanced MRI is related to the severity of Parkinson's disease

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Parkinsons disease (PD) results from degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc), leading to dopamine (DA) depletion in the striatum. This depletion is thought to alter neuronal activity in the basal ganglia, resulting in various symptoms including psychomotor ones such as bradykinesia, rigidity, and tremor. However, the pathological state of PD is associated with activity changes in which basal ganglia areas remains unknown. Here we show a correlation between striatal activity and the pathological state of PD using activation-induced manganese-enhanced magnetic resonance imaging (AIME-MRI). We found that compared to control mice, PD model mice showed significant changes in neuronal activity in the striatum. Moreover, striatal neuronal activity was significantly correlated with tyrosine hydroxylase (TH)-immunoreactivity in the striatum, which is related to motor performance in PD animal models. Thus, our results demonstrated that striatal activity is associated with the pathological state in PD. We think that our findings can pave the way for significant progress in research on PD pathophysiology, since AIME-MRI can be used for non-invasive investigation of whole brain activity. Our results also suggest that AIME-MRI could potentially be utilized for the study and diagnosis of various other neurological disorders

(COI: No)

P3-340

Effects of social isolation on the progression of allergic rhinitis symptoms in mice

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To clarify the relationship between allergic diseases and psychological stress, we investigated the effects of social isolation on the progression of allergic rhinitis symptoms in mice. Female BALB/c mice, aged 3 weeks, were divided into two groups: the group-housed and singly-housed groups. After 6 weeks, mice were sensitized by intraperitoneal injection of saline containing ovalbumin (OVA) and alum, as an adjuvant, on days 0, 5, and 14. Then, local sensitization was performed every day, starting from day 21, by instilling OVA in saline into the bilateral nasal cavities using a micropipette. From day 21, OVA-induced nasal symptoms were observed once every 2 days. Immediately after nasal instillation of the antigen solution into the bilateral nasal cavities, the frequency of sneezing and nasal rubbing was counted for 10 min. After symptoms of allergic rhinitis had progressed, a histamine H_1 receptor antagonist, epinastine, was administered intraperitoneally 60 min prior to the local application of antigen. The nasal symptoms induced by antigen solution were observed for 10 min. In singly-housed mice, the progression of allergic rhinitis was delayed and suppressed compared with group-housed mice. Treatment with epinastine decreased the nasal allergic symptoms in group-housed mice but not in singly-housed mice. These results show that the stress of social isolation partially inhibited the symptoms of allergic rhinitis and weakened sensitivity to epinastine.

(COI: No)

P3-341

Selective blockade of the cortico-rubral pathway masks the recovery of forelimb function by CIMT in capsular hemorrhage rats

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Forced-use of impaired upper limb, such as constraint-induced movement therapy (CIMT), is an effective rehabilitative method after stroke. We reported that CIMT after a capsular hemorrhage resulted in better functional recovery of the forelimb. However, the detailed mechanism of the recovery by CIMT is still unclear. To investigate the CIMT-induced changes of brain circuits and its causality for the recovery, Wistar rats were injected with collagenase to make internal capsule hemorrhage (ICH), followed by CIMT for 7 days from 24 hours after the lesion. As connection between ipsi-lesional motor cortex and red nucleus was enhanced in CIMT-treated ICH rats in biotin dextran amine (BDA) tracing analysis, double-virus vector infection technique was used to block the cortico-rubral pathway selectively (Kinoshita et al., 2012): NeuRet-TRE-EGFP. eTeNT was injected into the red nucleus and subsequent injection of AAV1-CaMKII-rtTAV16 at the motor cortex. It was revealed that blockage of the cortico-rubral tract by doxycycline resulted in deficits of the recovered forelimb function in CIMT-treated ICH group. Data suggest that cortico-rubral pathway is one of essential circuit for CIMT-induced recovery after ICH.

(COI: No)

P3-342

Roles of tPA on the recovery after ischemic stroke

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In ischemic stroke, neurons in the brain cause ischemic death which is associated with neurological dysfunction. This damage is recovered histologically by activation of microglia and astrocytes, and functionally via angiogenesis, remodeling of neural network and neurogenesis. Tissue plasminogen activator (tPA) has important roles on neural function and neuronal death in the brain together with thrombolysis in the vessel. Thus, we studied the roles of tPA on the histological and functional recovery after ischemic stroke. By using photochemically induced thrombosis model, a reproducible brain damage was induced in mice with or without tPA gene deficient (tPAWT or tPAKO) and assessed the neurologic functions by foot fall test, tail lift test and von Fray test, and histological responses by damage size and immunostaining of astrocytes and microglia. It was found that the retracation of damage size and the recovery of neurologic dysfunction assessed by tail lift test and von Fray test was delayed in tPAKO mice compared with tPAWT mice. Furthermore, the number of activated microglia was less in tPAKO mice than tPAWT mice. These findings indicate that tPA is involved in the improvement of histological damage and neurological dysfunction.

(COI: No)

P3-343

Effects of anti-cancer drugs on pain induction in a rat stomatitis model

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Stomatitis is frequently developed as a side effect of chemo-radiotherapy in head and neck cancer patients and induces severe pain during eating and speaking. In this study, to examine relationship between anti-cancer drugs and stomatitis-induced pain, we investigated effects of 5-fluorouracil (5-FU) and cisplatin on stomatitis-induced nociceptive behaviors in male Wistar rats. We intraperitoneally administered 5-FU (40 mg/kg, 3 times), cisplatin (4 mg/kg, 2 times) or saline (as control). After these administrations, rats were treated in the oral mucosa of the mandibular vestibule with 50% acetic acid for 30 seconds under pentobarbital anesthesia to develop stomatitis. Both anti-cancer drugs delayed healing from stomatitis and induced leukopenia, compared with control rats. 5-FU increased stomatitis-induced spontaneous nociceptive behavior, but cisplatin conversely inhibited it, likely anti-bacterial treatment. 5-FU exaggerated stomatitis-induced mechanical allodynia. On the other hand, cisplatin itself induced mechanical allodynia and further decreased mechanical withdrawal threshold was stomatitis induction. These results suggest that 5-FU exaggerates stomatitis-induced nociception due to bacterial overgrowth in stomatitis region. Additionally, cisplatin suppresses spontaneous nociception due to known anti-bacterial effect, but itself induces mechanical allodynia.

(COI: No)

P3-344

Antinociceptive effect of transcutaneous electrical nerve stimulation via an opioid mechanism in rats with adjuvant arthritis

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Objective: The aim of this study was to investigate the effects and mechanism of transcutaneous electrical nerve stimulation (TENS) in patients with chronic inflammatory pain.
Methods: Male Wistar rats were divided into four groups: the Control group, Adjuvant Arthritis rats (AA) group, TENS-treated AA rats (AT) group and TENS- and naloxone-treated AA rats (ATN) group. Arthritis was induced by the injection of complete Freund's adjuvant into the right hind paw. In the ATN group, naloxone, an opioid antagonist, (3 mg/kg, SC) was administered before the TENS treatment. The stimuli (4Hz, 30 min) were applied three times a week for two weeks, after which the mechanical and thermal pain thresholds were detected on days 0, 7 and 14 and the μ-opioid receptor (MOR) level in the spinal cord was analyzed immunohistochemically on day 14.
Results: On day 14, the pain thresholds were significantly decreased and the expression of MOR in the superficial part of the dorsal horn was increased in the AA group versus those observed in the Control group. These changes were inhibited by TENS treatment; however, the effects of TENS were attenuated by the administration of naloxone.
Conclusions: These results suggest that TENS treatment has an antinociceptive effect on chronic inflammatory pain in association with the endogenous opioid system.

(COI: No)

P3-345

Plasminogen Activator Inhibitor-1 Contributes to Glucocorticoid-induced Diabetes, Osteopenia and Sarcopenia in Female Mice

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Glucocorticoids (GC) treatment induces numerous adverse effects, including glucose/lipid abnormalities, osteoporosis and muscle wasting. However, its pathogenesis remains to be fully elucidated. The present study investigated the role of plasminogen activator inhibitor-1 (PAI-1) in GC-induced glucose/lipid abnormalities, osteoporosis and sarcopenia by using PAI-1-deficient mice. The levels of plasma PAI-1 and PAI-1 mRNA in white adipose tissue were markedly elevated in GC-treated wild-type female mice compared with placebo-treated wild-type female mice. PAI-1 deficiency significantly improved insulin resistance and glucose intolerance but not hyperlipidemia induced by GC treatment. In vitro study showed that active PAI-1 treatment attenuated insulin-induced phosphorylation of Akt in HepG2 hepatocytes, but not in 3T3-L1 adipocytes and C2C12 myotubes, indicating that PAI-1 inhibits insulin signaling in hepatocytes. PAI-1 deficiency blunted GC-induced bone loss presumably due to a decrease in apoptosis of osteoblasts. Moreover, PAI-1 deficiency protected from muscle loss induced by GC treatment. In conclusion, the present study indicated that PAI-1 is involved in GC-induced glucose metabolism abnormality, osteopenia and muscle wasting in female mice. PAI-1 may be a novel therapeutic target to reduce adverse effects of GC treatment.

(COI: No)

P3-346

Stress-induced microglial activation may be triggered by noradrenergic neurons

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Microglia has been extensively demonstrated to participate in the neuroinflammatory responses. Recent studies have shown that exposures of animals to stress, either acute or chronic, induce robust microglial activation in the brain. The stress-induced microglial activation has been well documented in the hippocampus, cerebral cortex, thalamus, hypothalamus, and substantia nigra. In the present study, we investigated the mechanism how acute stress could trigger microglial activation in the brain. For this purpose, we studied the spatial distribution of noradrenaline-synthesizing enzyme, dopamine β-hydroxylase (DBH), and activated microglial cells following 2 h period of restraint stress. The results demonstrated that: 1) the microglia activation, as demonstrated with Iba1, occurred in most of these brain regions including the hippocampus and substantia nigra; 2) DBH was densely stained in the neuronal fibers located in most of these brain regions including hippocampus and substantia nigra; 3) The intensity of DBH immunoreactive (IR) fibers and that of DBH-IR cell bodies in the locus ceruleus was significantly increased in the 2 h restraint stress; 4) β1 and β2 adrenergic receptor (AR) are co-localized with microglial cells; 5) The stress-induced microglial activation is significantly inhibited in the double knockout mice that specifically lack β1 and β2 AR. Thus, the present study demonstrates that neuron-microglia may have close interactions through noradrenaline throughout the brain. Noradrenaline may be one of the neurotransmitters that regulate microglial activation in the brain.

(COI: No)

P3-347

Effects of intraperitoneal injection of vasopressin on Oxidative stress in conscious rats

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It has been already known that the d-ROM of oxidative stress had been elevated by some kinds of invasion, example for surgical operation, inflammation, ischemia. However, It has not been shown that the physiological and psyclic stress induce the change of d-ROMs or not. Other hand, It has been also shown that the vasopressin can induce the emesis and the excitation of sympathetic activities. The aims of this study were to evaluate the change of oxidative stress [d-ROMs and BAP] under intraperitoneal administration of vasopressin for sympathetic excitation.7-week-old male Sprague-Dawley rats were divided into two groups each of seven control group and vasopressin group. Rats ate the 1.5 g solid food within 10 min after fasting for 24 h. After feeding, the control group was injected the saline (2 ml/kg) and other group war injected the vasopressin (20 μg/kg;2 ml/kg) with intraperitoneally. The stomach was excised 90 min after feeding, the ratio of gastric emptying was calculated from the weight of the contents. No changes had been obtained in this study value of d-ROM and/or BAP in vasopressin group were not significant compare to control group. It had been demonstrated that changes of oxidative stress were not observed under vasopressin injection in conscious rats. From that finding suggest that the sympathetic excitation by vasopressin in injection(i.p.) might not induce the changes of oxidative stress.

(COI: No)

P3-348

Acupuncture related to the vestibular system improves arterial pressure response at the onset of head-up tilt

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Acupuncture has been used for treating multiple kinds of disorders. However, the precise mechanism of acupuncture on such disorders remains unclear. We examined the effects of acupuncture related to the vestibular system for arterial pressure change at the onset of head-up tilt (HUT) in 14 healthy young subjects. Arterial pressure was measured continuously during supine position for 5 minutes, followed by 2 minutes of 60 degrees of HUT with and without acupuncture. With acupuncture, 2 points were tested for each subject using stainless steel acupuncture needles (15mm long, 0.16mm in diameter, 10 mm insertion). One point was TE17 (called *Yifeng*), which is considered to treat inner ear disease. Another point was PC6 (called *Nei Guan*), which is considered to treat nausea and vomiting symptoms. The order was changed randomly. Without acupuncture, mean arterial pressure (MAP) increased or decreased less than 5 mmHg upon HUT in 7 subjects (UP group), however, MAP decreased more than 5 mmHg in 7 subjects (DOWN group). In UP group, no significant difference in MAP was observed between with and without any acupuncture. In DOWN group with acupuncture of TE17, however, the decrease in MAP was smaller than that without acupuncture and with acupuncture of PC6. Therefore, acupuncture of TE17 is considered to be useful for treatment of orthostatic hypotension.

(COI: No)

P3-349

Influence of implantation of slow release corticosterone pellets on the hippocampal neuronal cells in C57BL/6 mice

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The use of exogenously administered corticosterone to experimental animals has some validity to examine chronic stress-induced damage in the brain. In this study, we have examined the influence of treatment with corticosterone on the hippocampal neuronal cells using male C57BL6/J mice, by implanting slow release corticosterone (5 mg). In the control experiment, placebo pellets were implanted. Seven or 21 day treatment with corticosterone resulted in much reduced thymus weight, compared with the control. The rate of increase in body weight was also lowered significantly by the corticosterone treatment. On the 7 or 21 day after implanting the pellets, we anesthetized the animals with intraperitoneal injection of pentobarbital. In some animals, isoflurane was additionally inhaled. Then, we transcardially perfused the heads with 4% performaldehyde solution, and made the Nissl-stained hippocampal preparations. In the preparations from the corticosterone-treated animals, there have been observed pyknosis, and partially degenerated neuronal cells in the region of CA2, CA3 or dentate gyrus. However, similar changes were also observed in the preparations from the control animals. Some of the degenerated changes in the hippocampal neuronal cells might occur due to implantation of the pellet itself, as there is no visible degeneration in the neuronal cells in the preparation from non- treated animals.

(COI: No)

P3-350

CD200-CD200R interaction may play a role in the growth of rat experimental glioblastoma

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CD200 and it's receptor CD200R are a type 1 trans-membrane glycoprotein, and CD200/CD200R interaction transduces suppressive signals to immune cells with CD200R expression. In this study, we investigated the tumor growth and the survival rate of rats that underwent C6 glioma cell transplantation into their forebrains. As revealed by immunohistochemical staining, most endothelial cells of blood vessels in the tumor mass expressed CD200 and tumor associated macrophages (TAMs) expressed CD200R. These results suggest that CD200 on endothelial cells interacts with CD200R on TAMs, causing the macrophage polarization into M2 phenotypes. We have found the expression of a truncated form of CD200 that we call CD200S lacking some amino acid sequences of CD200. CD200S appears to disturb the interaction CD200/CD200R interaction. With an aim to elucidate what kinds of roles the interaction plays on C6 glioma tumor progression, we established rat C6 glioma cells stably expressing CD200 and CD200S using a viral vector. The established cells (C6 transfected with empty vector, CD200, or CD200S) as well as normal C6 glioma cells were transplanted into the brain of neonatal rats. Although the four types of cells did not show any significant differences in the proliferation rate, rats transplanted with CD200S-tranfected C6 cells survived for significantly longer period than the rats transplanted with other cell types. The results suggest that CD200/CD200R interaction aids the progression of glioblastomas.

(COI: No)

P3-351

Monocarboxylate transporter 4 is associated with acidification of synovial fluid pH and synovial fibroblast proliferation in rheumatoid arthritis

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Objectives: Synovial fluid pH is low in rheumatoid arthritis (RA); however, the precise mechanisms are unclear. Here we investigate the correlation between synovial fluid pH and the disease activity of RA. We reveal the mechanisms regulating synovial fluid pH.
Methods: The pH and lactate concentrations in synovial fluid from RA patients were determined. Synovial fibroblasts (SFs) from the inflamed joints of RA patients (RASFs) were examined for the expression of ion transporters that regulate intracellular pH. The ion transporter up-regulated in RASFs was then suppressed by small interfering RNA (siRNA) and the effect of transfection was investigated.
Results: Synovial fluid pH correlated inversely with both the disease activity score using 28 joints and C reactive protein (DAS28-CRP) and synovial fluid lactate levels. RASFs had significantly higher mRNA and protein levels of monocarboxylate transporter (MCT) 4 than osteoarthritis SFs (OASFs). Knockdown of MCT4 induced RASF apoptosis and inhibited their proliferation, but not OASFs.
Conclusion: RA activity correlated with decreased synovial fluid pH. This may be due to increased MCT4 expression in RASFs. Since silencing MCT4 induced RASF apoptosis and inhibited their proliferation, MCT4 may be a potential therapeutic target for RA.

(COI: No)

P3-352

Development of rat diabetic nephropathy is suppressed by voluntary exercise in OLETF rats

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Purpose: The aim of this study was to examine whether voluntary wheel-running (WR) exercise suppresses development of diabetic nephropathy in OLETF rats of a type II diabetes mellitus (DM) model.
Methods: Male OLETF rats of 5 weeks old were reared in cages equipped with wheels (OLETF-WR) or in standard cages (OLETF-SED) for 16 months. These rats underwent urine collection in a metabolic cage, examination for HbA_{1c} and ELISA for renal injury biomarkers.
Results: Creatinine, BUN and urine volume per day were increased in OLETF-SED with high HbA_{1c} as compared with OLETF-WR and LETO. In addition, OLETF-SED and OLETF-WR showed the highest and moderate levels in both kidney/BW ratio and excretions of albumin and total protein into urine, respectively, whereas LETO the lowest levels. ELISA of nephron segment-specific injury indicated that OLETF-SED has injury in both glomerulus and proximal/distal tubules while OLETF-WR has slight injury just only in glomerulus.
Conclusion: Long-term voluntary WR exercise could suppress development of type II DM but not fully diabetic nephropathy.

(COI: No)

P3-353

Fibrinogen gamma-chain peptide-coated, ADP-encapsulated Liposomes Rescue Lethal Blast Lung Injury Hemorrhage via Purinergic Signaling

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Background: Fibrinogen gamma-chain (HHLGGAKQAGDV, H12)-coated, adenosine-diphosphate (ADP)-encapsulated liposomes [H12-(ADP)-liposomes] that accumulate at bleeding sites and release ADP. The aim of the study was to elucidate the effect and the mechanism of H12-(ADP)-liposomes on resuscitation of lethal blast lung injury.
Methods: Mice were pretreated with H12-(ADP)-liposomes, (ADP)-liposomes, (PBS)-liposomes or normal saline, and then received a single shot of Laser Induced Shock Wave (LISW) that caused diffuse alveolar hemorrhage.
Results: H12-ADP-liposomes significantly improved mouse survival and reduced the pathological injury score than normal saline (35 vs 40, p=0.004, n=5). H12-ADP-liposomes reduced the albumin leakage (0.8 vs 1.3 mg/ml, p=0.03, n=6) and MIP-2 levels in the bronchoalveloral lavage fluid (BALF) (74 vs 355 pg/ml, $p<0.01$, n=6) than normal saline. In this setting, exogenous ADP derived from the H12-(ADP)-liposomes not significantly up-regulated the platelet aggregation but was soon metabolized to Adenosine, which has cytoprotective effect.
Conclusion: H12-(ADP)-liposomes may be a safe and effective for acute blast lung injury via hemostatic support and drug delivery system of purinergic signaling for organ protection.

(COI: No)

P3-354

Licarin A is a candidate compound for the treatment of immediate hypersensitivity via inhibition of mast cell activation

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The present study evaluated the pharmacological effects of licarin A, a compound isolated from various plants, on A dinitrophenol-human serum albumin (DNP-HSA)-stimulated rat mast cell line (RBL-2H3). Licarin A (1 - 20 μM) significantly and dose-dependently reduced tumor necrosis factor α (TNF-α) secretion (IC_{50} 12.6 ± 0.3 μM) from DNP-HSA-stimulated RBL-2H3 cells. Furthermore, the secreted levels of prostaglandin D2 (PGD2) of DNP-HSA stimulated cells pretreated with licarin A were lower than those of cells stimulated with DNP-HSA alone (positive control). Treatment with licarin A at 20 μM revealed subtly suppression of the DNP-HSA-induced increase in cyclooxygenase-2 mRNA and protein levels. In contrast to its striking inhibition of TNF-α and PGD2 release, 20 μM licarin A only moderately inhibited histamine release from DNP-HSA-stimulated RBL-2H3 cells, by 28.2%, as compared with the positive control. We identified several signaling pathways which mediate these pharmacological effects. Licarin A treatment reduced phosphorylated protein kinase C alpha/beta II (PKC α / β II) and p38 mitogen-activated protein kinase (MAPK) protein levels. Taken together, our results demonstrate that licarin A reduces TNF-α and PGD2 secretion via the inhibition of PKC α / β II and p38 MAPK pathways, suggesting it may serve to attenuate immediate hypersensitivity.

(COI: No)

P3-355

Rare sugar D-psicose (D-allulose) prevents progression and development of diabetes in T2DM model OLETF rats

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Prevalence of obesity has emerged as life-style-related health problem leading to insulin resistance followed by T2DM. To cope with increased insulin demand pancreas β-cells become injured and failure followed by glucose intolerance. This circumstance demands balanced food intake. We introduce a zero-calorie sweet-taste food additive, D-psicose (also called D-allulose), a rare sugar, have been evaluated effective against hyperglycemia and hyperlipidemia, and represents a safe and non-toxic compound to maintain blood glucose levels through pancreas β-cell preservation in OLETF rats. Treated rats were fed 5% D-psicose. Control OLETF and non-diabetic control, LETO were fed water only. Body weight, food and drink, blood glucose and insulin were measured periodically. Oral glucose tolerance test was performed. Serum and organs were preserved, measured and stained. D-psicose controlled abdominal fat accumulation and prevented body weight increase. D-psicose improved insulin resistance through constant maintenance of blood sugar levels. Oral glucose tolerance test showed reduced blood glucose levels suggesting improvement of insulin resistance. D-psicose attenuated β-cell fibrosis. Serum levels of proinflammatory and antiinflammatory adipocytokines were also controlled well. Rare sugar D-psicose might be a promising strategy for the prevention of obesity, maintenance of blood sugar, and preservation of pancreas β-cells.

(COI: No)

P3-356

Induction of cell death in human cancer cell lines by novel small molecule activator of tyrosine kinase receptors

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Tyrosine kinase receptors (RTKs) mediate a variety of growth factors. Occasionally, RTKs are constitutively activated in malignant cells. Activation of RTKs are correlated with malignant progression of human cancer. Therefore, ATP competitive inhibitors for RTKs have been developed. We identified novel small molecule compound CMB-236 during anticancer drug screening. We observed that CMB-236 markedly induced elevation of kinase activity of a variety of tyrosine kinase receptors, using a TR-FRET kinase assays. In spite of elevated RTKs activity, CMB-236 induced cell death in human cancer cell lines using MTT assay. Significantly elevated caspase-3, -8, and -9 activity was observed in MDA-MB-231 breast cancer cells treated with CMB-236. The cell death induced by CMB-236 was prevented by simultaneously adding of pan-caspase inhibitor Z-VAD-FMK. We found that cell death induced by CMB-236 was strongly dependent on caspase activity. The cell death of MDA-MB-231 breast cancer cells treated with CMB-236 was decreased by combination of CMB-236 with sunitinib, a multitargeted tyrosine kinase inhibitor. Hence, we assumed that unusual activation of tyrosine kinase by small molecule compound disturbed cell homeostasis resulting in induction of cell death. It remains to be determined whether kinase activator will be efficient as a cancer therapy in vivo, but we suggest that such compounds can be considered as potential drug targets.

(COI: No)

P3-357

The development of whitening peptide with peptide percutaneous drug delivery system

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Topical therapy is the most favored form of treatment for whitening against hyperpigmentation and sunburn because it lends itself to self-administration, patient compliance and an absence of systemic adverse effects. However, high-molecular-weight, hydrophilic chemicals are difficult to use as transdermal delivery drugs and the use of topical drugs has been highly limited. Melanogenesis inhibitors from natural sources have great potential, as they are considered to be safe and largely free from adverse side effects. We applied 11-arginine (11R), a cell-membrane-permeable peptide, as a transdermal delivery system with a skin delivery enhancer, pyrenbutyrate. We performed intracellular screening for melanogenesis inhibitors with 11R fused with 28 kinds of tyrosinase inhibitory peptides from natural sources. Peptide No. 10, 8 amino acid, found in gliadin protein, a wheat component, most strongly inhibited melanin production, showed no cytotoxicity and inhibited melanin synthesis as determined through melanin content measured using an absorption spectrometer and observation with a transmission electron microscope. Next, we transduced this 11R-No. 10 into skin with an 11R transdermal delivery system after previous treatment with pyrenbutyrate and performed daily repetitive topical application for two weeks against a UV-induced sun-tanning guinea pig model. We observed a whitening effect and significant melanogenesis inhibition in a model skin sample by Masson-Fontana staining.

(COI: No)

P3-358

A novel methotrexate derivative with intrinsic magnetism

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Background: We have recently reported a novel anti-cancer compound with intrinsic magnetism (EI236). In addition to anti-cancer effect, EI236 has three features 1) EI236 is attracted by a magnet, i.e., magnetic drug delivery, 2) generating heat in an alternating current magnetic field, i.e., hyperthermic effect, and 3) a new contrast agent in magnetic resonance imaging (MRI), because of its magnetism. Based on these properties of EI236, we succeeded in generating a novel methotrexate derivative with intrinsic magnetism (m-MTX). It is well known that MTX is a commercially available and has been used as conventional drug for cancer and rheumatic diseases. In this study, we examined whether m-MTX has an intrinsic magnetism and the anti-cancer effect.
Materials & Methods: The magnetic property of m-MTX was measured by ESR (Electron Spin Resonance) and SQUID (Superconducting Quantum Interference Device). VX2, rabbit squamous cancer cells and MCF7, breast cancer cells, were used. To evaluate the m-MTX-induced cytotoxity, cell proliferation was measured using commercially available kit (ATCC).
Results: m-MTX was easily accumulated by a permanent magnet in water. ESR and SQUID showed that m-MTX has an intrinsic magnetic property. Furthermore, m-MTX inhibited cell proliferation in both cells in a dose dependent manner.
Conclusion: M-MTX may enable us to develop novel strategies in cancer treatment, i.e., chemotherapy with controlled drug delivery with a single drug compound.

(COI: No)

P3-359

Analgesic effect of hangeshashinto on oral ulcer-induced pain in rats

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It is well known that oral pain in head and neck cancer patients treated with chemo-radiotherapy is persistent and intractable, resulting under-nutrition and low quality of life. Recently, it has clinically reported that hangeshashinto (HST), a traditional Japanese medicine, is effective on the oral pain. However, mechanism of the analgesic effect has not been well known. In this study, we investigated the oral ulcer-induced pain and efficacy of HST to the pain in rats using new technique to apply direct stimulations in the oral mucosa. Treatment with acetic acid in the labial fornix region of the inferior incisors developed obvious oral ulcer. Application of HST to oral ulcer region did not change pain-related grooming behavior, suggesting that HST does not have pungent effects. Head withdrawal threshold to mechanical stimulation to the oral mucosa was decreased by oral ulcer development compared to naive. The decrement of mechanical threshold was recovered to naive level from 30 min to 60 min after topical application of HST to the oral ulcer region. These results support that HST is a useful drug to inhibit oral ulcer-induced pain in patients with chemo-radiotherapy.

(COI: Properly Declared)

P3-360

Evaluation of the antinociceptive effect of Uncariae Uncis cam Ramulus (Chotoko) in rat pain models

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Objective: Kampo medicine Yokukansan has been reported to be effective against neuropathic pain, such as that observed in patients with postherpetic neuralgia, central pain and trigeminal neuralgia. The aim of this study was to investigate the antinociceptive effects of Uncariae Uncis cam Ramulus (Chotoko), a crude drug component of Yokukansan on pain.
Methods and Results: 1) Acute study using formalin-induced pain model rats. In the Chotoko-treated group, Chotoko was administered for three days, after which 50 μl of a 5% formalin solution was injected into the right hind paws and the time spent licking the injected paw was recorded. The pre-administration of Chotoko resulted in a decrease in the licking time.
2) Chronic study using the chronic the constriction injury (CCI) model rats. The CCI model rats were prepared according to the model proposed by Bennett. Two weeks postoperatively, a decrease in the pain threshold in the CCI rats was confirmed and then Chotoko had been administered for two weeks. Four weeks postoperatively, the pain threshold significantly decreased and significant spinal astrocytic activation, which is involved in the expression of chronic pain, was noted in the CCI rats. However, the activation of astrocytes was controlled and the decrease in the pain threshold was reduced with the administration of Chotoko.
Conclusions: We therefore conclude that Chotoko can effectively reduce acute inflammatory and chronic neuropathic pain.

(COI: No)

P3-361

Effect of in vivo melanogenesis of Hyugatouki (Angelica tenuisecta var. furcijuga) extract

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Purpose: Angelica furcijuga (AF) is an endemic species and perennial herb of Japanese parsley department that grows wild in the Kyushu island in Japan. It is faced with extinction but its usefulness is attracted attention by success of organic grow. We have ever reported that the AF extracts from lobe and stem, stimulated melanogenesis in mouse B16 Melanoma Cell (B16 cell) and mouse hair, and effect of melanogensis in fraction of water and ethyl-acetate layer. In the present study, on the effects of the each extract solvent fraction of the AF's extracts from lobe and stem, melanogenesis of mouse hair were observed.
Methods: Before and after apllications, change of the melanogenesis of the back hair of the mouse with AF extract was investigated.
Results and Discussion: We observed different effects of each fraction on melanogenesis. This study suggests the certain fraction of AF's extracts contains the melanin production promoting substances.
(COI: No)

P3-362

Effect of pregabalin or pentazocine on restriction of movement and hyperalgesia in an ankle-immobilization rat model

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To evaluate the effect of pregabalin or pentazocine treatment on range of motion (ROM) limitation and the anti-hyperalgesia in ankle-immobilization model. Wister male rats were used. ROM and pain threshold were measured in all rats once a week for the 2 weeks. ROM of ankle dorsiflexion (DF) was measured, and pain thresholds were evaluated by behavioral response with the von Frey test and Hargreaves Assay using a plantar test. All data were shown as % of right limb/left limb. Ankle DF in ankle-immobilization for 14 days (IM) was significantly limited 2 week after immobilization (66%). And Ankle DF in IM+pregabalin (IM+PG) and IM+pentazocine (IM+PZ) also significantly limited 67% and 66% after 2 weeks, respectively. The mechanical hyperalgesia threshold in IM was significantly decreased to 39% after 2 weeks compared to control group, and in IM+PG was also significantly decreased to 58% respectively, but in IM+PZ was not decreased. Thermal nociceptive thresholds were significantly decreased to 61% after 2 weeks in IM, and also significant decreased 82% after 2 weeks in IM+PG, but in IM+PZ was not decreased. These results indicate that PG or PZ treatment was not effect on ROM of ankle DF in this model. On the other, the decrease of mechanical hyperalgesia and thermal nociceptive thresholds in IM+PZ were not observed, and showed a tendency to increase by IM+PG. It might act on central nerve system and/or spinal interneuron. We need further investigation for resolution these mechanisms.
(COI: No)

P3-363

Effectiveness of using specific arrow shapes in illustrations showing lipoprotein dynamics

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Animations showing the basic dynamics of lipoprotein, and then illustrations using specific arrow shapes for movement, changes, and facilitation were presented to 2nd yr students in nutrition, who had learned the topic previously. Results of an anonymous survey were as follows. Compared to other illustrations NOT using specific arrow shapes, for understanding, the presented ones were: easier 35, somewhat easier 30, no different 9, somewhat more difficult 0, more difficult 0. To "get the image" (comprehend the whole idea), the presented ones were: easier 39, somewhat easier 27, no different 8, somewhat more difficult 0, more difficult 0. Regarding memorization, the presented ones were probably: easier 28, somewhat easier 31, no different 15, somewhat more difficult 0, more difficult 0. The high evaluation indicates that presenting animations and then illustrations using specific arrow shapes is effective in showing the basic dynamics of lipoproteins.
(COI: No)

P3-364

Vestibulocochlear organ 3D print model generation trial

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It is very hard for medical students to dissect vestibulocochlear organs and to grasp their complex structures, partially because they are embedded in the temporal bone. We recently developed transparent temporal bone 3D-print-model with vestibulocochlear organs inside(The 119th Annual meeting) Our model worked well as a guidance of whole vestibulocochlear organ dissection. As a guidance of each of vestibulocochlear organs dissection, we developed auditory ossicles (malleus, incus, stapes) models in transparent composition of internal and external acoustic meatuses, and vestibulocochlear organs Since CT value oriented editions of DICOM data images did not always match anatomical structures, the human head CT scan images was edited manually slice by slice based on anatomical features. For example, mastoid cells are topologically identical to middle ear. So, they had to be removed manually. Then auditory ossicles data and surrounding structure data were saved independently to cast them with different resins. The edited DICOM data were then converted to STL data. To determine appropriate size of the model for beginners, double and triple sized models were generated by lamination of 0.2mm thick layers of ultraviolet curable resin with CONNEX 500 3D printer. The new model could be a good guidance for dissection of the vestibulocochlear organs.
(COI: No)

P3-365

Osteology on "Seikotsu Sinsyo", Judo-therapy book at Edo era(1810)

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The present study describes "SeikotsuSinsho" and Kagami Bunken. "SeikotsuSinsho" is a book of Judo-therapy that was written in 1810 by Kagami Bunken. The author was a Judo-therapy doctor of the late Edo period (1755-1819). In this book, general osteology, name of the bone, forms and function of each bone, joints and ligaments have been described. In addition, "KakuKotsu ShinKei Zu", which is a breakthrough skeletal atlas, is a precise sketch of skull, vertebrae, thorax, upper limbs, and lower limbs. The figures of these bones were sketched by Kagami Bunken based on the real bones. "SeikotsuSinsho" has been well known to Judo therapist, but not to Anatomist. We reviewed the terminology of osteology that has been described in this book in the documents from Edo era to early in the Meiji era. There were three types of the terminology of osteology found in "Seikotsu Shinsyo". (1): Terminology used by past orthopedic science, (2): the terms considered to have referred to the KaitaiShinsho, (3): the original terms of Kagami Bunken. However, the original terms have not been reflected in the current terms. As the reason for this, it is suggested that a translation term became mainstream.
(COI: No)

P3-366

Research for characteristics of physiology education in the Saitama Prefectural University and development of efficient educational program for students of several different courses of health sciences

Tanaka, Ken-ichi (*Physiol Pharmacol, Sch Health Social Serv, Saitama Pre Univ, Koshigaya, Japan*)

In Saitama Prefectural University (SPU), we have consistently provided quality education which helps graduates to play important roles in the area of health sciences. Moreover, we have to educate simultaneously basic medical sciences including physiology because we have several different department of health sciences such as nursing or physical therapy. Thus, we have tried to clarify characteristics of physiology education in SPU and development of efficient program of cross-sectional education for students of several different courses of health sciences using by questionnaire method. Firstly, we would analyze characteristics or tendency of needs of physiology education for each department of subjects divided according to specialty in the field of health sciences. Particularly, we would examine the effective cooperation method of three kinds of education subjects of lecture, practice and training in physiology education because we hope to be able to understand an effectual cooperation method among three kinds of educational methods. Data analysis are under way.
(COI: No)

P3-367

The "Hand-made" heart model as an educational tool for three-dimensional cardiac anatomy

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Background: The three-dimensional (3D) anatomy of the heart can be difficult to teach for teachers and to understand for students due to its complexity. We therefore developed a method to simulate the 3D cardiac anatomy using two hands.
Method: Our model is created by folding the two hands together as follows; (1) Make fists using the right and left hands with the thumbs and index fingers straight (a gun gesture). (2) Bring the two hands together to cross each other so that the left index finger is located between the right thumb and the right index finger. (3) Fold the thumbs onto the fist with the right thumb is placed over the left thumb.
Results: The hands together provide a simulated heart view with the cardiac base facing the examiner. The right and left hand represent the right and left heart, respectively. For each hand, the fist and index finger represents the ventricle, and great vessel, respectively. The base of the left and right forefinger correspond to the aortic and pulmonic valves, respectively. The left middle finger and thumb simulates the mitral valve. The base of the right thumb corresponds to the tricuspid valve. The border created by the two fists represents the ventricular septum. Major coronary branches are represented by anatomical landmarks of the hands and borders between the two hands.
Conclusion: Our model represents the cardiac geometry and normal distribution pattern of the coronary artery branches fairly accurately. We propose this model as a useful tool for both self-learning and education on the 3D cardiac anatomy.

(COI: No)

P3-368

Usefulness of fetal pig for understanding orofacial anatomy in anatomical practice

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In recent years, we have known as much about the difficulties of the learning about the anatomical knowledge, especially in orofacial region of oral anatomical education for dental technician and dental hygienist students. We have already introduce the usefulness of fetal pig for understanding tooth development and dental germ in anatomical practice in the 115th and 119th Annual meeting of the Japanese Association of Anatomists. Anatomical practice as a mandatory elective subject using fetal pigs has already been conducted in the first year for two academic years at our school. In the present study, students who took the anatomical practice course using fetal pigs in 2012 and 2013 were asked to complete a questionnaire in order to determine if their level of understanding of anatomy, particularly in orofacial anatomy, had increased or not.

(COI: No)

P3-369

Trial of Anatomical Education by Anatomical Tour Using a Rotation Method for Pharmaceutical Students

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Anatomical tour is an educational method using dissected cadavers mainly for comedical students. In a previous study, we have documented the anatomical tour by using a rotation method is effective in getting good emotion for healthcare students and medical/pharmaceutical graduate students (Master course). In this study, we tried the anatomical tour for pharmaceutical undergraduate students, and their impressions were surveyed by a questionnaire. Half of the pharmaceutical students (n=43) were divided into three groups, and each group attended observational learning at 3 cadavers by rotation (15 min × 3); concerning (1) thoracic viscera and upper limbs, (2) abdominal viscera, and (3) dorsal structures including lower limbs and brain/spinal cord. Finally they observed all cadavers freely for 30 min. Another half of the students (n=44) attended the tour 1 week later. Following impressions were obtained by the questionnaire (n=85); the anatomical tour is very satisfactory (59%), or fairly satisfactory (36%). These data suggest that anatomical tour using the rotation method is also effective in anatomical education for pharmaceutical undergraduate students.

(COI: No)

P3-370

Anatomy Education in the Visual Science Course

Tsujikawa, Hiroshi; Kadoya, Koji (*Fac. Med. Sci. & Welf., Tohoku Bunka Gakuen Univ., Sendai, Japan*)

In the Visual Science Course of Tohoku Bunka Gakuen University, we study and teach visual sciences as well as we train students for orthoptists (ORT). The purpose of this presentation is to discuss anatomy education in our course. In education for ORT, subjects about visual organ are dominant, needless to say. However, anatomy and physiology of the whole body are also required because the national license exams for ORT include the area of this study, for example. Among subjects in our curriculum, "Introduction to Anatomy and Physiology I and II" for first year student, "Structure and Function of Sense Organs" and "Seminar for Structure and Function of Sense Organs" for second year student contain anatomy education, respectively. In addition, fourth year students can select anatomical themes for "Graduation Research". In "Seminar for Structure and Function of Sense Organs", students study several area of anatomy, such as dissection of eyeballs of pig, observation and drawing of histological specimens by using a microscope, macro-anatomy by using an anatomical model of human body, study of human skull and skeleton, somatometry and surface anatomy in eye and its surrounding area, for instance. In "Graduation Research", several students supervised by one of the presenters, have studied ocular anatomy such as, vascular system, extraocular muscles and orbital osteology by dissecting fatal pigs for the past three years.

(COI: No)

P3-371

Functional model of Swallowing

Satoda, Takahiro[1]; Ikuta, Natsumi[2]; Minoda, Memori[2]; Shimoe, Saiji[1] (*[1]Grad. Sch. Hiroshima Univ., Hiroshima, Japan; [2]Hiroshima Univ. Sch. Dentistry Student*)

It is difficult to teach students about the mechanism of swallowing. There are three phases of swallowing; oral phase, pharyngeal phase and esophageal phase. The bolus of food is propelled to back of mouth by the tongue and the swallowing reflex happens. After nasopharynx and mouth closure, the glottal closure occurs, then hyoid and larynx are lifted by the contractions of suprahyoid and thyrohyoid muscles. As for the epiglottis, it is compressed by the tongue and inclines downward. As the larynx is lifted upward and antriorly, slight vacuum is caused in the lower pharynx and upper esophagus at the same time, and pharyngeal constrictor compress bolus, therefore, the bolus passes the piriform fossa, and is inhaled into the esophagus. This time, we made a model in order to explain this complicated mechanism. We used three sliding rails. One rail is fixed to the top of backbone horizontally and added two hooks, one hook is for the tongue movement and the other lifting the soft palate. The other two rails are fixed to the backbone vertically and the both tops of these rails are connected by using wire in order to push down the posterior rail and lift up the anterior rail. Wooden chip fixed to posterior rail represented the contraction of pharyngeal constrictor. Hyoid bone and larynx connected to anterior rail can be lifted by pushing down the posterior rail. The mandible is made of paper clay by using a metallic plate in it. The tongue, the soft palate, and the epiglottis are made by using the EVA (Ethylene Vinyl Acetate) sheet. Suprahyoid, thyrohyoid muscles are made of tube using wire in it.

(COI: No)

P3-372

Extraction of narrative intention in medical documents based on morphological analysis

Shimmi, Takahiko; Tatsumi, Haruyuki (*Grad. Sch. Med., Sapporo Medical Univ., Sapporo, Japan*)

In general, documents as the "reification of thoughts, " compound of wording elements such as noun, verb, auxiliary verb, modifier, adjective, post-positional particles, endings and so forth, conforming with its semantic relation and specific stemma. This indicates that the structure of documents and capability/functionality of words are available to approach in morphological (=anatomical) standpoint. Concretely, analysis on words-meanings relations and paragraphs shows its “context.” Moreover, context and frequently-appeared word extract the “stressed intention of author.”In this study, morphological analysis is implemented on elements and functionality of documents. Based on the analysis, finally, it suggests that the relation between structure and intention are on mutual-supplement: the extraction of intention is possible to be described on the course of context.

(COI: No)

P3-373

Positional relationship between the nerve and vascular of drawing blood, intravenous injection site in the cubital fossa using ultrasonic echo equipment

Gomi, Toshiaki[1]; Hirata, Miwa[2]; Kimura, Akihiko[1]; Naruse, Hideo[1]; Yajima, Hiroyoshi[1]; Nishihara, Ken[3]; Terajima, Miho[4]; Isho, Takuya[5]; Ishikawa, Youichi[6]; Sasaki, Seizou[7]; Hayashi, Hiroyuki[3] (*[1]Tokyo Ariake Univ. Med. Healt. Sci, Tokyo, Japan; [2]Tokyo Healthcare Univ., Tokyo, Japan; [3]Saitama Prefec. Univ., Saitama, Japan; [4]Univ. of Tsukuba, Tsukuba, Japan; [5]Takasaki Gener. Hosp., Takasaki, Japan; [6] Dep. Anat. Toho Univ., Tokyo, Japan; [7] Orient. Med. Resear., Tokyo, Japan*)

Purpose: Drawing blood, intravenous injection in the cubital fossa has been carried out on a daily basis, but complications such as nerve and vascular damage is occurring frequently. This time, the positions were examined about the blood vessel nerve bundle (median nerve (N), brachial artery (A) and brachial vein (V)) that existed in the injection site deeply with a ultrasonic echo equipment.
Method: 202 were examined. It took pictures of the Huter line (cubital fossa horizontal line). The distance and depth of N, A, and V from the middle point of the base line to the ulnar side (inside) were investigated.
Result: The distance and depth from the middle point of the base line to N, A, and V are each 19.1±5.53mm, 8.0±2.17mm, 16.1±5.43mm, 6.6±1.94mm, 16.7±6.28mm, 6.6±2.13mm. As the position of NAV, 6 patterns of NAV, NVA, ANV, AVN, VNA, VAN were seen from ulnar side (inside) to cephalic side (out side).
Conclusion: The distance, depth, and each position to N, A, and V that composed the blood vessel nerve bunch in cubital fossa was obtained. Basilic vein and median basilic vein that exists on the inside(ulnar side) of the cubital fossa, with caution when we puncture them.

(COI: No)

P3-374

Creating electronic materials for the effective teaching of anatomy courses

Inomata, Reiko; Kamezawa, Hajime; Komazaki, Shinji (*Saitama Med. Univ., Saitama, Japan*)

In order for our country to develop and compete internationally, a qualitative change in education is recommended. To this end, we propose that traditional teaching methods be converted to newer, more efficient ones such as active learning and flipped learning. Concurrently, in order to create a foundation for educational reforms, electronic teaching materials should be created or updated and effective teaching methods should be developed using these materials.
In this presentation, we introduce some simple methods for producing practical electronic materials for teaching anatomy courses by using high-functioning free software that is widely available throughout the world. We designed electric teaching materials using our methods, and explore new teaching methods such as active learning using our teaching materials. Descriptions of our electronic materials for teaching anatomy courses and our findings from trials of the new teaching techniques used are included.

(COI: No)

P3-375

RealEEG: a toolkit for medical students' training on EEG recording and analysis

Matsuzaka, Yoshiya (*Dept Physiol, Grad Sch Med, Tohoku Univ, Sendai, Japan*)

An ideal training of electrophysiology for students would be that every student is given the chance to directly experience the recording of bioelectric activity from neuromuscular system. Yet in reality, the high cost of commercial instruments for physiological experiments often precludes the purchase of sufficient number of units, thereby limiting the number of students who experience the recording of neural and/or muscular activity in group teaching. Therefore, I developed a low-cost toolkit for students' training on electrophysiology. The toolkit includes a custom designed amplifier with adjustable gain and two poles band pass filter. It is designed to function even in electrically unshielded environment due to the differential amplification with driven-shield inputs. Further, a PCB (printed circuit board) of this amplifier was developed for easy replication with consistent quality. Using this amplifier, I built a computer-controlled closed loop stimulation and measurement system for the training of electroencephalography (EEG) recording by medical students. Using this system, students succeeded in recording various brain activity with clarity which otherwise would have required costly instruments. The technical information to replicate this resource is freely available for educational as well as research purposes in other institutions.

(COI: No)

P3-376

3D multi-depth dissection atlas as a complementary resource for anatomy education

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Here we report an anatomical education trial of "MeAV Anatomie," a 3D multi-depth, multi-angle dissection image display system, developed by Panasonic and Okayama University. Anatomy dissection is the most effective method to master in-depth human morphology. Students appreciate human structures by hands-on training of dissection as well as studying textbooks and atlases. The dissection is, though, an irreversible process that makes iteration learning virtually unrealistic. This also means that students have no second chance when challenging difficult structures to dissect. Although photographic anatomy atlases and 3D human models may compensate such difficulties to some extent, these would not cast realistic, detailed 3D structures. MeAV Anatomie includes sets of photographs, or "contents, " of different anatomical regions. A cadaver was dissected to series of depth levels, and photographed at each level from multiple angles around the hemisphere to reproduce a 3D stereoscopic dataset. One can view the cadaver images from arbitrary angles and dissection levels on PCs or iPads. We installed a client PC system with a 3D display in the dissecting room, and distributed an iPad with the viewer app to each dissection group. Students could use the system for their homework, redeeming the irreversibility problems of dissections. They could also use it to simulate dissection procedures during actual dissections. We used some of the contents for lectures and examinations. The academic effects and students' perspectives were reviewed to estimate the validity of the system.

(COI: No)

P3-377

The brain networks underlying the Velvet Hand Illusion: an fMRI Study

Rajaei, Nader[1]; Kitada, Ryo[2]; Aoki, Naoya[2]; Takahashi, Haruka[2]; Miyaoka, Tetsu[3]; Ohka, Masahiro[1]; Sadato, Norihiro[2] (*[1]Dept. Complex Systems Science, Grad Sch Information Science, Nagoya Univ, Nagoya, Japan; [2]National Institute for Physiological Science; [3]Shizuoka Institute of Science and Technology*)

when humans put their hands together with grids of wires between them and wires move, they perceive illusory sensation of velvet between wires (velvet hand illusion, VHI). The VHI has attracted considerable attentions from the engineers for its application to haptic virtual reality. However, it is poorly understood about its underlying neural mechanisms. We experience the VHI by interpreting tactile inputs originated from velvet, even though the inputs are actually originated from wires and skin of the hand. Therefore, the VHI would involve the two brain networks, one related to perception of velvet surface per se and the other that are involved in grouping and segregating tactile inputs. To test this hypothesis, we conducted a functional MRI experiment wherein 30 subjects went through the following four conditions: strong VHI, weak VHI, real velvet and baseline condition involving no stimulation with wires. The contrast of strong VHI (against weak VHI) and the contrast of real velvet (against baseline condition) both activated the postcentral gyrus (PostCG) and cerebellum. By contrast, strong VHI produced greater activation than real velvet in the intraparietal sulcus (IPS) and precentral gyrus (PCG). This result indicates that the IPS and PCG are involved in misinterpretation of tactile inputs, the PostCG and the cerebellum are related to perception of velvet in the VHI.

(COI: No)

P3-378

Effects of the body composition on physiological changes after the Judo match

Takashina, Terue[1]; Kushi, Hidehiko[1]; Fukada, Kihachiro[1]; Onuma, Naoko[1]; Amano, Kiichirou[1]; Yoshida, Akira[2]; Konno, Jun[3] (*[1]Graduate School of Literature and Social Sciences, Nihon University, Tokyo, Japan.; [2]Institute of Humanities and Social Sciences, Nihon University, Tokyo, Japan.; [3]College of Humanities and Sciences, Nihon University, Tokyo, Japan.*)

Introduction: It is reported that body fat percentage increases with increasing weight class, with a specially rapid increase in the >90kg classes. The purpose of this study was to compare physiological changes before and after a Judo match between men < 90kg and >90kg.
Subjects and Methods: This study included 15 men who were divided into 2 groups: one is" < 90kg (n=7)"and ">90kg (n=8)". Body composition (Body fat, skeletal muscle and extracellular water percentages) was measured before the match, while heart rate, blood lactate, tympanic temperature, and skin temperatures were measured before and 1, 10, and 20 min after the match.
Results: Body fat percentage was significantly higher in the >90kg than in the < 90kg. Skeletal muscle and extracellular water percentages was significantly higher in the < 90kg than in the >90kg. Blood lactate were higher in the >90kg than in the < 90kg significantly at 1, and 10 min after the match. Skin temperatures were higher in the >90kg than in the < 90kg significantly at 10 min after the match.
Conclusions: This study revealed that Judo athletes with lower skeletal muscle, extracellular water and higher body fat percentages accumulated more blood lactate and a skin temperatures fall is delayed after the Judo match.

(COI: No)

P3-379

Effects of Trunk Training on Weight-Lifting Performance

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Introduction: Tests were performed to examine whether trunk training improved weight-lifting performance.
Subjects and Methods: The subjects were 30 high-school students (grades 10-12) who were weight-lifting club members. Eight exercises were selected from the trunk training program of university gymnastics club. The subjects trained for 2-4 months, 6 times a week. Before and after the training program, the subjects snatch (S) and clean and jerk (C&J) weight-lifting performances were measured. Changes in the measurements, including growth rate, were compared between the subjects with and those without trunk training.
Results: The results of the trunk training program were as follows: The recorded levels of improvement for the training group were 6.64 ± 1.57 kg for the S lift and 6.68 ± 1.50 kg for the C&J lift, while those for the non-training group were 0.63 ± 0.38 kg and 1.75 ± 0.67 kg, respectively, indicating significant improvements for the subjects who underwent trunk training.
Conclusions: Through this study, the significant contribution of trunk training on the improvement of weight-lifting performance was clarified.

(COI: No)

P3-380

"Info-Medicine" : the Center of Innovative Concept Based on an Anatomical View Point

Tatsumi, Haruyuki; Mizoguchi, Shogo; Shimmi, Takahiko; Sakaki, Fusako; Ninomiya, Takafumi; Ichikawa, Ryoichi; Kikuchi, Shin; Ohta, Shuzo (*Grad. Sch. Med. Sapporo Medical Univ., Sapporo, Japan*)

We define "information" as multimedia stimuli, which move our mind. The mind is the brain function composed of brain cells. Generally speaking, external as well as internal stimuli change the cell status. Some pattern of stimuli becomes a signal, and then information. Therefore, appropriate and timely information exerts therapeutic effects on the cells of our body in addition to our mind. Taking advantage of this nature of the multimedia stimuli, we could develop good medicine in order to make human healthy. So we have coined the concept of "Info-Medicine (Info-Med)." According to this definition, conventional drugs, gene therapies, psychotherapies and Tsubo (acupuncture point) stimulations also come under the scope of "Info-Med". From the points of cell biology, socio-psychology, and "The Theory of Moral Sentiments" written by Adam Smith, we developed "Info-Med" in a much broader sense and proposed "Full-Powered Medicine" utilizing everything good for the health, in contrast to the modern medicine, which is partial. We classified the Info-Med into five types:In-Social, To-Brain, In-Brain, To-Cellular, and In-Cellular. The center of the innovative concept is that "So you believe, you believe so", like "Cogito ergo sum" by Descartes. We have made a proposal of "Strategic Defensive Medical-Care Initiative "(SDMCI)" named after Ex-President Regan's SDI (Strategic Defense Initiative) taking advantages of IT (Information Technology) going to realize the SDMCI by full-powered medicine with "Info-Med".

(COI: No)

P3-381

Sphingosine 1-phosphate receptor-2 plays a protective role against lipopolysaccharide (LPS)-induced acute lung injury

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Sphingosine 1-phosphate (S1P) is a lysophospholipid mediator and plays an important role in the regulation of vascular barrier function. Recently we demonstrated that in a murine anaphylaxis model, S1P receptor-2 ($S1P_2$) plays a protective role against vascular leak, hypotension and lethality through inhibiting endothelial nitric oxide synthase (eNOS). Acute lung injury (ALI) is characterized by leukocyte infiltration into the lung parenchyma, pulmonary vascular permeability increase and edema, and resultant pulmonary dysfunction. However, the role of $S1P_2$ in ALI is still unknown. Here, we explored the role of $S1P_2$ in a murine model of ALI induced by intra-tracheal administration of LPS. $S1P_2$ deletion in mice aggravated leukocyte infiltration in the lung parenchyma, elevation of protein concentrations and neutrophils in bronchoalveolar lavage fluid, and increases in lung proinflammatory cytokine mRNA expression. $S1P_2$ deletion also aggravated LPS-induced increases in vascular permeability and pulmonary edema. Administration of NOS inhibitor, Nω-L-nitro-arginine methyl ester, inhibited exacerbation of leukocyte infiltration and vascular hyperpermeability in $S1P_2$-deleted mice. These results suggest that S1P2 plays a protective role against LPS-induced ALI possibly through inhibiting NOS and is a novel therapeutic target for ALI.

(COI: No)

P3-382

Linking genotype to phenotype in mice molars by means of morphometric mapping

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The mouse dentition has been extensively used as a model for the developmental genetic basis of dental morphology. Phenotypic change and disorder have been reported in a variety of mutant mouse strains. In the case of mutant mice showing drastic morphological change in cusp patterns, however, the conventional quantitative approaches, such as landmark-based methods, cannot be used due to the lack of biologically and/or geometrically homologous structures between specimens. Therefore, the phenotype-genotype correlation has remained to be clarified. Here, we applied methods of morphometric mapping (MM), a homology-free method for characterizing the phenotype, to analyze the coronal morphological variation of molars in two strains of wild type mice: ICR and BL6, and two types of mutant mice: CSGalNAcT1 (Chondroitin sulfate N-acetylgalactosaminyltransferase1, a key enzyme for CS synthesis) -null and Msx2-null. Our data showed that the MM enabled to discriminate not only between wild type and mutant, but also between two wild type strains with precise quantification and visualization of the complicated crown surface morphology. Applying this method to various types of mouse mutants representing altered cusp pattern promises well for an elucidation of the genotype-phenotype mapping in more details.

(COI: No)

P3-383

Nucleoprotein affect on Cell-Cycle progression in human cancer cells

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We had previously reported that a nucleoprotein (NP) has an effect of growth suppression for human cancer cells in vitro. We had also showed that NP affected on cell cycle progression of cancer cells, especially delayed the shift to G2/M phase. The event would contribute to the growth suppression of cancer cells. This time, we analyzed the global gene-expression patterns to reveal the molecular mechanism in the anti-cancer effect of NP using human breast cancer cells, MCF7. As a result, presence of NP affected the expression of many genes, 145 and 111 genes were up and down-regulated, respectively. VDR (vitamin D receptor) and CDKN1A (cyclin-dependent kinase inhibitor 1A), reported as cancer prevention, were up-regulated. Moreover, antioxidant enzyme GPX2 and GPX8 (glutathione peroxidase 2 and 8) were also up-regulated. These studies provide a possibility that NP will suppress the growth of cancer cells through such a change of gene expression.

(COI: No)

P3-384

Anti-tumor p53 fagment peptides screening and treatment effect of transdermal delivery for malignant melanoma

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Intracellular delivery with Protein Transduction Domain (PTD) is rapidly evolving methodology in vitro or in vivo. We have established "protein therapy" with poly-arginine (11R) as PTD, which has been employed to transport various bioactive molecules into cells. So far, based on this method, we have applied poly-arginine (11R) to transdermal delivery system, as an application example, skin whitening and hair growth agents. Such direct and efficient "Transdermal approach" may be also effective in skin cancer (malignant melanoma). In this study, we tried to establish novel peptide therapy with transdermal approach for malignant melanoma. Recently, several paper showed p53 fragment peptide had anti-tumor effect in vitro and in vivo. In this time, we found some anti-tumor peptides from p53 fragment peptide library screening. One of them strongly induced apoptosis and inhibited human melanoma cell proliferation. Furthermore, we tried to administrate this peptide against melanoma mouse model with peptide transdermal approach.

(COI: No)

P3-385

Action of peripheral opioid to gastric emptying under peripheral acute inflammation induced by carrageenan injection on foot pad in rats

Hamamoto, Kentaro[1]; Taniguchi, Sazu[2]; Taniguchi, Hiroshi[3]; Katoh, Singo[1]; Takeshima, Chiaki[1]; Isaji, Keiyu[1]; Okoda, Misaki[1]; Taguti, Reina[2]; Itoh, Kazunori[2]; Kitakoji, Hiroshi[2]; Imai, Kenji[2] (*[1]Meiji University of Integrative Medicine Graduate school; [2]Meiji University of Integrative Medicine Department of Clinical Acupuncture and Moxibustion; [3]Meiji University of Integrative Medicine Department of Basic Acupuncture and Moxibustion*)

The aims of study were to investigate the action of peripheral opioid to gastric emptying under peripheral acute inflammation induced by carrageenan injection on foot pad in rats Thirty male Sprague-Dawley rats were divided into three groups (each of 10), that were control (as vehicle) group, subcutaneous injection of carrageenan group, and carrageenan+naloxone methiodide group, respectively. All study had been demonstrated in fasting period after 20 hours from final feeding. At 4 hours after the injection to foot pad, rats were gave the solid food with 1.5 g and they ate food within 10 minutes. The stomach was excised 90 min after feeding, to evaluate gastric emptying from the weight of the contents. Percent of gastric emptying were calculated from the dry weight of contents after 72 hours, and that were compared among three groups. Ratio of gastric emptying in the carrageenan-treated group had been significant lower than control group. Therefore, the remarkable changes of gastric emptying in the carrageenan-treated were rivaled by the administration of naloxone methiodide. From these findings suggest that gastric emptying have been delayed by the β-endorphins in the peripheral blood by the carrageenan injection.

(COI: No)

P3-386

Action of peripheral opioid to oxidative stress under peripheral acute inflammation induced by carrageenan injection on foot pad in rats

Imai, Kenji[1]; Hamamoto, Kentaro[2]; Taniguchi, Sazu[1]; Taniguchi, Hiroshi[3]; Ueda, Naoki[1]; Katoh, Singo[2]; Takeshima, Chiaki[2]; Isaji, Keiyu[2]; Okada, Misaki[2]; Taguti, Reina[1]; Itoh, Kazunori[1]; Kitakoji, Hiroshi[1] (*[1]Meiji University of Integrative Medicine Department of Clinical Acupuncture and Moxibustion; [2]Meiji University of Integrative Medicine Graduate school; [3]Meiji University of Integrative Medicine Department of Basic Acupuncture and Moxibustion*)

Aims of this study were to investigate the action of peripheral opioid to oxidative stress under peripheral acute inflammation induced by carrageenan injection on foot pad in rats Thirty male Sprague-Dawley rats were divided into three groups (each of 10), that were control (as vehicle) group, subcutaneous injection of carrageenan group, and carrageenan+naloxone methiodide group, respectively. All study had been demonstrated in fasting after 20 hours from final feeding. The blood was collected from the heart after 5.5 hours after the intervention. Using free radical analysis system, measured the redox ability and oxidative stress from the collected blood. And, pain threshold had been measured by Randall selitto at before and 4 hours after carrageenan injection. In addition, the foot circumference and body temperature had also been measured. Ratio of oxidative stress in the carrageenan-treated group had been significant higher than control group. Further, the carrageenan+naloxone methiodide administration group had shown more remarkable increase than the carrageenan-treated group. From these results suggest that the oxidative stress is elevated under the acute inflammation on limbs that are controlled by the opioid.

(COI: No)

P3-387

Effects of press tack needle treatment in rats subjected to chronic social isolation

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Objective: The aim was to investigate the effects of press tack needle (PTN) treatment on social isolation stress and the participation of orexin A in this effect.
Methods: Male rats were divided into a non-stress group (Control), stress group (Stress) and stress plus PTN treatment group (PTN). The rats in the PTN and Stress groups were housed alone for eight days. In the PTN group, a PTN (Pyonex, Seirin Co., Japan) was fixed on the GV 20 acupuncture point (Baihui) on day 7. We measured the stress behavior based on the time the rats spent biting a wooden stick for ten minutes on days 7 and 8 and measured the plasma corticosterone levels on day 8. In addition, the plasma orexin A levels and morphology of the hypothalamic orexin neurons were investigated on day 8.
Results: On day 8, the biting time and the plasma corticosterone levels were significantly increased in the Stress group versus the Control group, although these increases were inhibited in the PTN group. Meanwhile, the plasma orexin A levels and number of hypothalamic orexin neurons were significantly increased in the Stress group versus the Control group, and these increases were also inhibited in the PTN group.
Conclusions: PTN may inhibit the response to social isolation stress. The inhibitory effects of the secretion of hypothalamic orexin are thought to be one mechanism underlying this phenomenon.

(COI: No)

P3-388

Blood glucose peak time after meal in healthy population using CGMS profiles

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Introduction: Continuous glucose monitoring system (CGMS) provides an opportunity to better understand abnormalities in glucose metabolism in both patients with diabetes. The purpose of the study is to clarify whether postprandial glucose peak time and peak value using CGMS among healthy subjects.
Methods: Thirteen healthy female volunteers were divided into two groups: under 50 year-old and over 50 year-old. The glucose levels of 13 healthy volunteers were monitored over 24 hours and required to maintain their usual life style without any limitation. We measured the postprandial glucose peak time, which was defined as the time elapsing from the start of the meal to the highest recorded glucose value. We compared the mean of the peak time between the meal periods.
Results: The peak time of postprandial interstitial glucose concentration in under50 years vs. over 50 was 31.88 ± 10.9 vs. 60.80 ± 28.6 minutes (breakfast), 50.50 ± 17.2 vs. 66.80 ± 31.5 minutes (lunch), and 60.00 ± 22.3 vs. 89.60 ± 49.4 minutes (dinner), respectively. The postprandial glucose peak time in the over-50 group was significantly longer than the under-50 group. But, no significant difference was found in the peak value of postprandial glucose between the both groups.
Discussion: The result indicates that the postprandial glucose peak time and peak value was different for the different meal period. In conclusion, this study demonstrates that the postprandial glucose peak time and peak value differs in the age bracket.

(COI: No)

P3-389

Immunohistochemical examinations of the ganoine in regenerated scales from Lepisosteus oculatus, an actinopterygian fish

Sasagawa, Ichiro[1]; Ishiyama, Mikio[1]; Yokusuka, Hiroyuki[1]; Mikami, Masato[1]; Oka, Shunya[1]; Uchida, Takashi[2] (*[1]Nippon Dental Univ., Niigata, Japan; [2]Grad. Sch. Biomed. Sci. Hiroshima Univ., Hiroshima, Japan*)

It is necessary to compare teeth with scales during investigations of the origin of teeth in vertebrates because the dentition is considered to have arisen from skin denticles (scales). In basic actinopterygians, a well mineralized ganoine layer, which is analogous to tooth enamel, is present on the surface of ganoid scales [1]. In Polypterus, the preganoine (ganoine matrix) was found to exhibit immunoreactivity for anti-mammalian amelogenin antibodies, as has been found for collar enamel [2, 3]. However, there are no data about the preganoine in Lepisosteus (gar). In this study, the preganoine of regenerated Lepisosteus scales was immunohistochemically examined using anti-mammalian amelogenin antibodies. Positive immunoreactivity for several of the antibodies was detected in the preganoine, suggesting that the Lepisosteus preganoine contains amelogenin-like proteins that are similar to those found in tooth enamel [4, 5].

[1] Sire, J.-Y. (1994) Anat Rec, 240, 189.
[2] Zylberberg, L. et al. (1997) Anat Rec, 249, 86.
[3] Sasagawa, I et al. (2012) Cell Tissue Res, 347, 369.
[4] Ishiyama, M. et al. (1999) Arch Histol Cytol, 62, 191.
[5] Sasagawa, I et al. (2014) Connect Tissue Res, 55, 225.

(COI: No)

P3-390

The management of secondary infection control in gross anatomy education in Shimane University Faculty of Medicine

Ogawa, Noriko[1]; Hirano, Satoru[1,2]; Yokota, Shigefumi[2]; Matsumoto, Akihiro[1]; Furuya, Motohide[1]; Oka, Tatsuro[2]; Yasui, Yukihiko[2]; Otani, Hiroki[1] (*[1]Dept. Dev. Biol., Fac. Med., Shimane Univ., Izumo, Japan; [2]Dept. Anat. and Morphol. Neurosci., Fac. Med., Shimane Univ., Izumo, Japan*)

For gross anatomy dissection, we embalm cadavers by formalin infusion and substitution to alcohol for preservation. However, there is no solid evidence that this method completely prevents secondary infection. Whereas the infection control manual has been established in the affiliated hospital, there has been no manual for accidental infection in the Faculty of Medicine, Shimane University. Therefore, we established procedures and the manual in the Faculty of Medicine. 1. To decline an offer of cadaver with records of active specific infectious diseases such as viral hepatitis. 2. To examine upon arrival of all cadavers for specific infections such as type B or C hepatitis, HIV infection and HTLV-1 infection. 3. To cremate cadavers without embalming when infection detected. 4. To use disposable products such as surgical gowns. 5. To antiseptice instruments using sodium hypochlorite or autoclaving. 6. We prepared the manual for accidental infection in the Faculty of Medicine. Almost all hospitals carry out infection controls these days. Infection control should be equally performed in Faculties of Medicine for workers and students. We here present the management of infection control in our Faculty of Medicine, and recommend staffs of anatomy departments in Japan to be aware of the need for secondary infection control.

(COI: No)

Joint Program on Education

Joint Program on Education 1

Educational Lecture

(March 22, 9:00～10:30, Room B)

EP1-1 Neurobiology of axon: membrane excitation and propagation

Kamiya, Haruyuki Dept Neurobiol, Grad Sch Med, Hokkaido Univ, Sapporo, Japan

EP1-2 Educational Strategy of Basic Medical Science: for Students' "Deep Learning"

Kobayashi, Naoto Sch Med, Ehime Univ, To-on, Japan

Joint Program on Education 2

Model Lecture

(March 22, 16:00～18:30, Room B)

EP2-1 "Physiology Educator" accredited by Physiological Society of Japan

Nakashima, Akira Dept Physiol Chem, Sch Med, Fujita Health Univ, Toyoake, Japan

EP2-2 Chemical control of respiration

Suzuki, Atsuko Lab Physiol, Health Science Univ, Yamanashi, Japan

EP2-3 Anatomy of the lower limbs

Murakami, Tohru Gunma Univ Grad Sch Med, Maebashi, Japan

EP2-4 Feature detection in visual systems

Inokawa, Hitoshi Department of Physiology and Systems Bioscience, Kyoto Prefectural University of Medicine, Kyoto, Japan

EP2-5 Functional anatomy for understanding the swallowing mechanism

Abe, Shinichi Tokyo Dental College, Tokyo, Japan

EP2-6 To improve your lecture

Matsuo, Osamu Kinki Univ Faculty of Med, Osakasayama, Japan

Joint Program on Education 3

Educational Lecture

(March 23, 9:00～10:30, Room B)

EP3-1 Cortical development: what we have learned from the mouse and the human brains

Sato, Makoto Osaka Univ. Grad. Sch. Med., Suita, Japan

EP3-2 Cardiac electrophysiology (electrocardiogram), arrhythmias

Furukawa, Tetsushi Dept Bio-informational Pharmacol, Med Res Inst, Tokyo Medical and Dental Univ, Tokyo, Japan

Joint Program on Education 4

Educational Lecture

(March 23, 13:30～15:00, Room B)

EP4-1 Anatomical and physiological tradition in the Western medicine

Sakai, Tatsuo Juntendo Univ. Grad. Sch. Med., Tokyo, Japan

EP4-2 Research Ethics and Conflict of Interests (COI)

Kurata, Kiyoshi Dept Physiol, Hirosaki Univ Grad Sch Med, Hirosaki Japan

Joint Program on Education 5

Educational Lecture

(March 23, 15:00～17:15, Room B)

EP5-1 Immune system and lymphoid organs: to understand basic structures for the body defense

Matsuno, Kenjiro Sch. Med. Dokkyo Med Univ, Tochigi, Japan

EP5-2 Basic properties of biological membranes

Fujimoto, Toyoshi Grad Sch Med Nagoya Univ, Nagoya, Japan

EP5-3 Physiology of human growth

Koibuchi, Noriyuki Dept Integrative Physiol, Gunma Univ Grad Sch Med, Maebashi, Japan

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Poster Presentations

Joint Program on Education

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The Joint Meeting of
the 120th Annual Meeting of The Japanese Association of Anatomists
the 92nd Annual Meeting of The Physiological Society of Japan

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