Anxiety and Future Thinking: Effects of Emotion on Repeated and Alternative Episodic Future Event Simulation

Research Director: Dr. Daniel L. Schacter

Anxiety is an emotional state that can have both beneficial and detrimental effects on day-to-day functioning. Low levels of anxiety can reflect an important capability to adapt and plan for the future, whereas pathological anxiety often leads to incorrect yet inflexible predictions of aversive future events that can negatively impact social relations and individual well-being. Recent evidence has revealed that the imagination of emotional future events involves the fronto-limbic brain circuit, the same circuit that is fundamentally disrupted in anxiety disorders and that has been linked to the cognitive control of emotional content. These overlapping neural correlates suggest that anxiety may influence the ability to simulate hypothetical emotional future scenarios, a relationship that has received little attention within existing literature. The present studies sought to address the gap in the current literature using behavioral and neuroimaging techniques. First, we utilized a novel future event simulation paradigm to examine the relation between anxiety and emotion in simulating alternative versions of the future. We found that trait anxiety was positively associated with the amount of extra time it took participants to simulate alternative emotional, but not non-emotional, future events after a previously simulated event had been retrieved from memory. The results of this first experiment illuminate the difficulties that anxious individuals have with emotional control in the context of future event simulation. Secondly, we made use of a repetition suppression functional magnetic resonance imaging paradigm in order to more clearly delineate which brain regions serve to represent emotional and non-emotional simulations of the future. We expect that the results of this experiment will help to identify specific regions of interest for understanding the effects of repeating or alternative simulations of the future in anxious populations.

Association of Socioeconomic Status with Prefrontal Cortical Thickness and Subcortical Volumes in Children and Adolescents

Research Director: Dr. Margaret Sheridan

Socioeconomic status (SES) is a composite of several social and economic variables that is associated with physical and mental health, as well as cognitive development. SES predicts language abilities and success on tasks of attention, inhibition, and working memory. These deficits suggest SES may be associated with the structures in the brain that are thought to be necessary to language and executive functions. However, few studies have sought to correlate SES and brain structure. I aim to characterize the relationship between SES and cortical thickness in 225 typically developing children and adolescents aged 8 to 17 using magnetic resonance imaging (MRI). The cortical thickness of several regions of interest (ROIs) is calculated using T1 weighted MR images reconstructed and segmented in the FreeSurfer MRI processing software. If changes in cortical structure mediate the association between SES and deficits in language and executive functions, then an association between SES and prefrontal cortex thickness is expected. Associations with cortical thickness in other regions are not hypothesized and have been inconsistent in previous studies.

Behavioral and electrophysiological study of social and nonsocial orienting in children at-risk for ASD, A

Research Director: Dr. Charles A. Nelson

Autism spectrum disorder (ASD) is a developmental disorder diagnosed on the basis of impaired social and communicative abilities and the presence of restricted and repetitive behaviors. In addition to sociocommunicative deficits, ASD is associated with the atypical development of attentional processes. Understanding more about the emergence of atypical attentional processes and the development of neural mechanisms that distinguish children with ASD from typically developing (TD) children may lead earlier diagnosis and the emergence of more efficacious interventions. This study examined attentional orienting to social and nonsocial information in children at-risk for autism (HRA). Thirteen children between the ages of 4 and 6 (LRC = 7; HRA = 6) participated in a behavioral orienting paradigm and a complementary auditory oddball experiment, which measured attentional orienting to social and nonsocial stimuli. Behavioral results showed that LRC children oriented significantly more to social compared to nonsocial stimuli, whereas the HRA children showed no significant difference in orienting patterns. Electrophysiological data paralleled these findings with respect to both amplitude and latency of the P3a response. The LRC group oriented to social stimuli quicker and with greater amplitude than to nonsocial stimuli, while the latency and amplitude of the HRA group across conditions was not significantly different. These findings suggest that the HRA children have impaired social orienting, which may represent an endophenotype for autism. Future studies employing larger sample sizes are necessary to confirm whether social orienting is uniquely impaired, and if impairments in attentional orienting are associated with a diagnosis for ASD.

Behavioral cues as triggers for aggression in Drosophila melanogaster

Research Director: Dr. Edward A. Kravitz

Aggression is a behavior present in almost every complex organism believed to have evolved in the context or obtaining or defending resources. Like males of most species, Drosophila melanogaster males court females and only attack other males, a process that is dependent on sex recognition. Recently, it has been shown that both pheromonal and behavioral cues are sufficient to trigger aggression in male Drosophila, since males attack females that either display a masculinized pheromonal profile or exhibit male patterns of behavior. However, the specific behavioral cues that are sufficient to elicit male aggression remain elusive.

The work of my thesis is focused on identifying how behavior acts as a trigger for male aggression. To explore this question I will study the interactions between wild type males and masculinized females, in which expression of the sex determination gene transformer in the nervous system has been silenced and as a result exhibit male-specific behavioral patterns of courtship and aggression. By scoring specific patterns of behavior, such as the frequency or latency of female attacks (lunges) or courtship events, I aim to find a correlation between a specific cue and the onset of male aggression. I hypothesize that the male's transition to aggressive behavior would be immediately elicited by a masculine display of aggression from the female. To test this, I am currently performing experiments using females that only display male patterns of aggressive behavior towards the males.

Unlike the behavioral response to pheromones, which is mediated by the chemosensory system, the response to the behavior of the other animal is likely to involve a learning process, since males eventually transition from courtship to aggression in the presence of masculinized females. To test whether males are able to learn, and eventually remember, that females can behave as opponents, I will analyze male behavior during subsequent social interactions with females.

Characterizing transcriptional regulation of the DYT6 dystonia gene, THAP1

Research Director: Dr. Cristopher Bragg

Dystonia is a movement disorder characterized by sustained, involuntary muscle contractions. DYT6, a hereditary form of primary dystonia, is linked to many different mutations in THAP1, which encodes the THAP1 DNA binding protein. Four single nucleotide polymorphisms (SNPs) were identified in the putative 5' promoter region, which appear differentially enriched in DYT6 versus control individuals. These SNPs are -236_237 GA>TT, -42 C>T, -40 T>C, and -32 C>T. Bioinformatics analysis has also identified that nanog, a transcription factor involved in cell pluripotency, may bind to the promoter of THAP1. The importance of the promoter region in the ultimate regulation and transcription of THAP1 makes the SNPs and nanog significant for further investigation.

To analyze the effects of the SNP variants on THAP1 expression, mutagenesis was used to introduce the polymorphisms into THAP1 cDNA clones. These cDNAs, driving a firefly luciferase gene, were employed in luciferase reporter assays to determine the relative effect the SNPs have on THAP1 expression. Nanog cDNA and three controls were also tested in these assays to analyze nanog's role in THAP1 transcriptional regulation. Lastly, EMSA's were utilized to identify binding interactions between nanog and THAP1.

Data indicated that -236_237 GA>TT causes diminished THAP1 expression as compared to wild-type in neuroblastoma cells. Additionally, the EMSA's suggested that nanog binds the THAP1 promoter, although further experiments are required to show specificity. These results implicate -236_237 GA>TT as a potential disease modifier in DYT6 dystonia. Furthermore, nanog may have a regulatory effect, suggesting a possible role of THAP1 in neurogenesis.

Clinical and neuropsychological correlates of diffusion tensor imaging indices in first-episode schizophrenia

Research Director: Dr. Martha E. Shenton

Although the etiology of schizophrenia remains unknown, diffusion tensor imaging (DTI) has provided support for the disconnectivity theory, as it allows for the investigation of white matter tract integrity. Abnormalities in specific tracts are likely to underlie the clinical symptoms and neuropsychological impairments seen in schizophrenia, and changes in different tracts may explain the clinical heterogeneity among patients. In this study, 30 first-episode schizophrenic patients and 30 healthy controls, group-matched for age, sex, and parental socioeconomic status, were scanned using a high-resolution DTI sequence in a 3T scanner and administered a neuropsychological battery. Patients were also evaluated for clinical symptoms using the Scale for the Assessment of Negative Symptoms and the Scale for the Assessment of Positive Symptoms. I used tractography, a specific DTI technique that defines a significant portion of a specified tract, to extract tracts that have been implicated in schizophrenia, including the cingulum bundle, uncinate fasciculus, inferior longitudinal fasciculus, and arcuate fasciculus/superior longitudinal fasciculus. In the right cingulum, fractional anisotropy (FA), a general measure of white matter integrity, was found to be lower in patients compared to controls. Partial correlation analysis revealed a negative correlation between the left cingulum trace and avolition-apathy ($p = 0.017$), a negative correlation between right cingulum FA and Wisconsin Card Sort total errors ($p = 0.029$), and a positive correlation between left uncinate trace and bizarre behavior ($p = 0.001$).These findings link symptoms of schizophrenia to the integrity of neural tracts, lending additional support to the disconnectivity theory of schizophrenia.

*Analyses have not yet been completed for all tracts

Discovering The Development Of The Blood Brain Barrier: Visualizing Gene Expression

Research Director: Dr. Chenghua Gu

Each organ of the body requires blood circulation to deliver nutrients and remove wastes. This is especially true of the brain whose energy metabolism is one of the highest in the body. Therefore, the study of the blood brain barrier during development was aimed at understanding some of the molecular components of tight junctions, which make up the blood brain barrier. This study started with the identification of potential genes through a microarray analysis of cortex and lung endothelial cells from E13.5. This list was compared against several online atlases to look for vascular patterns. After thirteen genes were indentified as potential candidates, their expression in the cortex of embryonic mice was investigated through immunohistochemistry. Western blots and qRT-PRC were also used to compare expression at different ages when antibodies were not readily available. Several genes were shown to have notable expression patterns, such as angiomodulin (AGM), solute carrier family 38, member 5 (Slc38a5), regulator of G-protein signaling 4 and 5 (RGS4; RGS5) and major facilitator superfamily domain containing 2 (mfsd2a). Specifically, mfsd2a has shown to be co-localized with tight junction markers, and mutant mice can be identified by “vessel leakiness” at E15.5. Further in vitro studies need to be conducted on all these potential candidates, but continuing experiments on mfsd2a look promising for identifying its function as related to the development of the blood brain barrier.

Early detection of Preclinical Alzheimer's disease with the Face Name Associative Memory Exam

Research Director: Dr. Dorene M. Rentz

Background:
Emerging research suggests the most optimal time to intervene in Alzheimer's disease (AD) is during the preclinical phase when amyloid beta (Aβ) plaques and other neurodegenerative changes are occurring years before the emergence of clinical symptoms. However, traditional cognitive tests have been insensitive to these early changes.

Objective:
The purpose of this study is to explore whether a sensitive associative memory test called the Face Name Associative Memory Exam (FNAME) is related to biomarkers of neurodegeneration including amyloid deposition, hippocampal volume (HV) and white matter hyperintensities (WMH). We hypothesize that performance on the FNAME will be related to amyloid deposition and hippocampal volume but may not be related to WMH.

Methods:
We examined 129 cognitively normal (CN) subjects (mean age = 73.7 ± 5 .9, education = 15.8 ± 2.9; AMNART IQ = 121.7±8.1) with Clinical Dementia Rating (CDR) scores = 0. All subjects received the FNAME, amyloid imaging with PET using Pittsburgh Compound B (PiB) and MRI scans. Using multiple linear regression analysis, we related FNAME scores to PiB retention (DVR, cerebellar reference), hippocampal volume, and white matter hyperintensities, co-varying for age and AMNART IQ.

Results:
A significant relationship for FNAME name retrieval (FN-N), controlling for age and AMNART IQ was found in relation to hippocampal volume (β=.000, p=.009)r and amyloid burden (β= -.856, p=.035; but not to white matter hyperintensities (β= .000, p=.222).

Conclusion:
FNAME performance is significantly related to amyloid deposition and hippocampal volume but not white matter hyperintensities suggesting that the FNAME is a sensitive measure of early AD pathology.

Effect of Striatal Deep-Brain Stimulation on Cognitive Recovery After Traumatic Brain Injury, The

Research Director: Dr. Emad Eskandar

Intermittent deep-brain stimulation (DBS) of the dorsal and ventral striatum was demonstrated to enhance associative learning and motivation, respectfully. However, these effects have yet to be determined in the context of traumatic brain injury (TBI), in which the brain's limited regenerative capacity can thwart the "relearning" necessary for reestablishment of proper cognitive function, thereby subjecting millions of patients to chronic cognitive deficits. Here, we present a study that seeks to determine the effectiveness of striatal DBS in ameliorating TBI-induced cognitive deficits. Mice were given TBIs via controlled cortical impact (CCI) and then implanted with electrodes either targeting the dorsal or both the dorsal and ventral striatum. To assess cognition, mice were subjected to various tasks in both the Morris Water Maze (MWM) and T-maze. Mice with dorsal-only electrode targets received chronic DBS for six weeks prior to cognitive examination, while mice with dorsal-and-ventral electrode targets received intermittent stimulation during cognitive examination. Mice in both stimulation schemes demonstrated greater cognitive recovery in comparison to non-stimulated mice, as shown by their ability to learn novel visuospatial associations in both the MWM and T-maze at quicker rates. Furthermore, this study also features the engineering of a novel-yet-ineffective extracranial adaptor to facilitate stereotactic electrode implantation, the successful development of a novel method to conduct electrophysiological experiments in the MWM, and the characterization of the motivational enhancement induced by ventral striatal DBS, namely that DBS motivates mice to persistently seek available rewards rather than preferentially seek larger rewards. Overall, despite the omission of few control groups and reliance on small samples, this pilot study outlines a framework for which basal ganglia DBS can be used to hasten and enhance rehabilitation after TBI.

Effects of Early Institutionalization on Emotional Face Processing, The

Research Director: Dr. Charles A. Nelson

Early institutional rearing has been shown to detrimentally affect the physical and neurological development of young children. children (Gunnar, 2001; Johnson, 2000; Zeanah et al., 2003). Deprived of social interaction fromin their earliest daysmonths of life, institutionalized children often display an abnormal neural responses to emotional faces, resulting in behavioral consequences such as the inability to understand and discriminate emotional expressions , and ultimately, deficits in social communication. This study examined differences in emotional face-processing abilities between three groups of children aged 11 years and 1 month to 13 years and 7 months, all residing in Bucharest, Romania: 27 who were raised since soon after birth in one of six institutions; 29 who were later removed from the institution and placed in high-quality foster care; and 23 who lived in the community and spent no time in institutional care. Event-related potentials (ERPs) were recorded while subjects viewed faces displaying emotional expressions of varying type and intensity. The amplitude of anthe early negative face-sensitive (N170) ERP component (N170) was then compared between the groups in response to expressions of both high and low intensities. Preliminary results revealed no significant group differences in mean N170 amplitude nor in right-hemisphere specialization, computed as the difference in mean amplitude between the chosen right and left hemispheric regions. Future Additional analyses will examine differences within the foster-care group based upon age of placement to investigate the possibility of a sensitive period in early social development.

Effects of Soluble Amyloid-β[beta] Peptides on Transmission and Plasticity in the Hippocampus

Research Director: Dr. Venkatesh N. Murthy

Alzheimer's disease (AD) adversely affects memory formation and storage, functions mediated by hippocampal plasticity. A hallmark pathology of AD is the presence of plaque deposits, which comprise oligomers of amyloid beta protein (Aβ1−42). Research shows high concentrations of Aβ1−42 disrupt hippocampal plasticity, but the effects on the network are unclear. To understand the molecular mechanisms of this disruption, I examined how Aβ1−42 affects a form of plasticity called long-term depression (LTD), and its general effects on excitatory and inhibitory currents in older animals. To test the hypothesis that Aβ1−42 acutely affects AMPA receptor trafficking and LTD, I studied the effects of 2.2 μM Aβ1−42 on a timescale of minutes. Whole-cell patch clamp recordings of hippocampal CA1 neurons in mice (8+ weeks old) showed that Aβ1−42 blocked LTD induction. Furthermore, Aβ1−42 did not alter the paired-pulse ratio of transmission, suggesting a postsynaptic mechanism for its effect on LTD. Next, field potential recordings revealed Aβ1−42 depressed basal transmission of CA1 synapses, suggesting occlusion—via the same molecular pathway—was responsible for the absence of LTD with Aβ1−42. Finally, any differential effect of Aβ1−42 on excitation versus inhibition can bear important consequences on CA1 transmission. To determine this effect, I calculated the average ratio of excitatory to inhibitory postsynaptic currents (E-I ratio). Interestingly, Aβ1−42 depressed inhibitory transmission more than excitatory transmission; therefore, the E-I ratio increased. Overall, these results laid the foundation for me to further investigate the role of Aβ1−42 on CA1 transmission and plasticity, and to test new hypotheses regarding the differential effects of Aβ1−42 on excitation and inhibition.

Exploring Multisensory Facilitation Using Functional Connectivity MRI in Humans

Research Director: Dr. Randy Lee Buckner

While sensory cues from different modalities are processed by distinct circuits, these environmental signals rarely appear to us in isolation. The combination of information from different sensory modalities can enhance our ability to detect and distinguish stimuli. Specifically, the combination of low-discriminability stimuli results in the highest level of enhancement. Although earlier studies predominantly support the presence of multisensory interactions in upper-level association centers, recent findings have challenged this viewpoint, providing evidence for multisensory integration in putatively unimodal areas. Here we used functional connectivity MRI (fcMRI) to study neural activity and connectivity while participants completed variants of a simple visual discrimination task. We found that reaction times to weak stimuli were significantly slower than to strong stimuli. Auditory cueing produced faster reaction times to visual stimuli, suggesting that the presence of the cue may facilitate detection or processing of the visual stimulus. We next localized brain regions responsive to auditory stimuli alone, visual stimuli alone or to both modalities. Across tasks, functional connectivity was observed to change as a function of the presence or absence of an auditory cue, notably in putatively unimodal sensory processing regions. Further analysis will test whether these connectivity changes occur between the unimodal regions themselves or predominately to multimodal regions in the brain. These results demonstrate that connectivity changes do not occur exclusively in higher-order association cortices, but rather that unimodal regions also dynamically change functional coupling during multisensory processing.

Fish food: neuromodulatory systems involved with feeding behaviors in larval zebrafish

Research Director: Dr. Florian Engert

According to the CDC, more than 35% of Americans are considered obese. In addition, more than 8 million Americans have eating disorders such as anorexia or bulimia. Therefore, it is of critical importance to understand the brain circuitry involved in our desire for food. We developed a behavioral assay for the food preference of larval zebrafish. Larval zebrafish track, chase, and eat unicellular protozoa called paramecia. We built a chamber that allows groups of fish to move freely throughout, but has paramecia only on one half of the tank. This offered the fish a choice between eating and not eating, depending on its location in the tank. We tracked their coordinates automatically with an infrared camera. We found that zebrafish consistently swam on the side of the tank that contained paramecia. Exposing larval zebrafish to serotonin antagonists abolishes this preference for food. Dopamine and opioid antagonists have no effect. Furthermore, single fish were observed for five hours in a small paramecia-containing dish. Recording with a high frame rate camera allowed us to distinguish and count individual paramecia over time. From this we could infer a rate of paramecia consumption. Preliminary results indicate that serotonin antagonists may also decrease the rate of prey consumption. These data indicate that the serotonergic system is intimately involved in prey capture and feeding behavior. We plan to chemically ablate serotonergic populations in the brain and examine the effects on behavior.

Investigating the fidelity of axolotl forebrain regeneration

Research Director: Dr. Jeffrey Macklis

While the mammalian central nervous system is characterized by an inability to regenerate following injury, many organisms possess remarkable regenerative capacity of neural tissue. The principal vertebrate species capable of regeneration is the paedomorphic salamander Ambystoma mexicanum, the Mexican axolotl, which reconstitutes spinal cord and brain following injury. Recent evidence has also demonstrated the presence of actively dividing matrix zones in dorsal and ventral regions of the adult forebrain. Considering that human CNS injury can result in irreparable, debilitating deficits in motor control, sensory processing, speech, and cognition, understanding the mechanisms responsible for neural regeneration has broad appeal for the field of regenerative medicine. As a long-term goal, such information might yield insight on how to initiate and direct regenerative response with molecular controls identified in naturally regenerating model organisms.

Here, we investigated the molecular identity of pallial neurons in the axolotl forebrain, characterizing progenitor/postmitotic neuron domains, and identifying the expression of subtype-specific molecular controls in the forebrain. We further analyzed these neurons on the basis of their projection patterns and dendritic morphology, using retrograde labeling and Golgi staining. In pursuit of future fate-mapping experiments, we have also standardized electroporation-mediated gene delivery in these organisms, and have successfully incorporated a reporter construct into ventricular zone cells in the pallium. Moreover, these characteristics are being used to assess the fidelity of regeneration at 2, 4, 6, and 8 weeks after the removal of one-third of the forebrain via mechanical injury. Taken together, these approaches demonstrate that the axolotl forebrain can be analyzed with molecular resolution, and data from ongoing regeneration experiments will be presented.

Investigating the role of the lateral habenula in reinforcement learning

Research Director: Dr. Naoshige Uchida

In order to survive in their respective environments, animals must learn to adapt. According to reinforcement learning models, such adaptation depends on reward prediction errors (RPEs), or differences between actual and expected reward. The lateral habenula is implicated in reward processing and encodes RPEs. To investigate whether the lateral habenula is necessary for learning from positive or negative outcomes, I compared the performance of control and habenula-lesioned mice in a classical conditioning tasks. I examined four different phases of the conditioning behavior: acquisition, extinction, relearning, and spontaneous recovery after extinction. The habenula lesioned mice showed faster extinction and slower relearning compared to non-lesioned control mice. However, further experiments are necessary in order to conclusively determine the role that the lateral habenula plays in learning behavior.

Mapping the Brain's Connections to Midbrain Dopamine Neurons Using Modified Rabies Virus in Transgenic Mice

Research Director: Dr. Naoshige Uchida

The human brain contains billions of neurons organized into circuits that process specific kinds of information and give rise to behavior. Information encoded by these circuits hold the key to understanding how the brain works and offer the promise of clarifying the causes of neurological and psychiatric diseases. Establishing improved methods to understand how neural circuits are connected is a critical step toward understanding how neurons communicate. In this project, I explored the advantages and limitations of Cre-mediated rabies virus retrograde labeling technology to investigate the connectivity of the midbrain dopamine system in mice. Dopamine neurons originating in the midbrain play pivotal roles in the brain, regulating motivation, movement control, and reward-seeking behavior. Recently, we have comprehensively identified the monosynaptic inputs to dopamine neurons. To elaborate and expand our knowledge of the dopamine system, I identified the sources of inputs at the di-synaptic level. Using this rabies-mediated retrograde tracing method, I investigated the di-synaptic inputs to dopamine neurons in the ventral tegmental area via the ventral pallidum, ventral striatum, and lateral hypothalamus, regions that are associated with high levels of monosynaptic input to the dopamine neurons. This project will demonstrate the utility and constraints of our methodology and contribute to our understanding of information transmission in the brain. Unraveling multi-synaptic input pathways for dopamine neurons provides a foundational knowledge in the regulation of dopamine neuron activity, and therefore a better neurobiological basis for the development of improved and targeted clinical interventions to improve the health of people and populations.

Meditate to Create: A Neurobiological View on the Effects of Mindfulness Meditation on Creativity

Research Director: Dr. Sara Lazar

Creativity is an extremely complex human behavior that requires a multitude of approaches to understand; yet it can be seen as one of humanity's fundamental elements as it allows humans to flourish throughout their lives. Recently, methods have been sought out to increase levels of creativity in all fields. Meditation should be able to induce states of higher creativity, as meditation is known to reduce levels of anxiety, fear, judgment, while increasing internal and external awareness, thus allowing for minimized inhibitions and the higher probability of new connections. Within this study, subjects were randomly assigned to participate in either an eight-week mindfulness meditation program that combines open-monitoring and focused-attention meditations, or an exercise program with a focus on nutrition. MRI brain imaging was conducted at the pre- and post- timepoints of the program. A standardized divergent thinking test (Torrance Tests of Creative Thinking) was administered at the pre- and post- timepoints, along with a battery of psychometric tests evaluating perceived stress, mindfulness, and creative achievement. Exploratory whole brain analyses as well as region of interest analysis showed that there were changes within the precuneus and regions in the temporal parietal junction for both meditators and exercisers, areas that have been linked to certain aspects of consciousness and creative thinking. CAQ scores were positively correlated with increased grey matter in the temporal parietal junction. Findings suggest that meditation and exercise can both lead to higher levels of creativity.

Neuro-Development in Post-Fontan Adolescents

Research Director: Dr. Michael J. Rivkin

Objectives: To investigate the development of sub-cortical structures in adolescents aged 10-18 who, as toddlers, underwent the Fontan procedure as palliation of the class of congenital heart defects known as Single Ventricle Defect (SVD) using volumetric magnetic resonance imaging (MRI).

Methods: 128 post-Fontan patients and 49 healthy control subjects were scanned using either a 1.5-Tesla or 3-Tesla MRI system. Sub-cortical volumes were differentiated by region and measured using the structural MRI analysis software Freesurfer, then subject to manual editing. Groups were compared using a multiple linear regression model.

Results: Of the 14 areas that constiutethe sub-cortical gray matter of the brain in both hemispheres, , 7 were significantly reduced in volume t in post-Fontan vs. healthy control subjects. The thalami, pallidi, and hippocampi bilaterally demonstrated reduced volumes, as well as the amygdala in the left hemisphere.

Conclusions: A volumetric analysis of parcellated sub-cortical gray matter revealed that post-Fontan adolescents demonstrated significant volume reduction as compared to healthy controlsin7 of the 14 component regions. These structural features of brain demonstrate that gray matter volumes are reduced among adolescents treated with the Fontan procedure for their congenital heart disease much earlier in life.

Observational Learning of Complex Procedural Tasks in Octopus Vulgaris

Research Director: Dr. Florian Engert

Comparative neurobiology can yield great improvements in our understanding of how complex behaviors emerge from neural processes. Though comparison of neural structure and ability has been richly studies between vertebrate species, the cephalopods, invertebrates of notable intelligence, have been the subject of much less comparative study. While the cephalopod brain and nervous system follows an invertebrate structure based on a central nerve ring and sub-order of ganglia, it's central ring is heavily cephalized into a large, multi-lobed brain replete with extensive optical lobes and a vertical lobe believed to be analogous to the vertebrate hippocampus. Using the common octopus, Octopus vulgaris, as a model I sought to elucidate some of the common cognitive capabilities shared between the cephalopod brain and the phylogenetically and structurally distant vertebrate brain. I examined the ability of O. vulgaris to engage in observational learning of procedures and concepts and how the vertical lobe is involved in mediating learning and memory of these tasks. Using a demonstrator-observer learning paradigm, I have shown that O. vulgaris is capable of learning simple procedural tasks via observation alone, from a combination of imitation of demonstrator methods and reduced neophobia resulting from conspecific interaction. Furthermore, I have shown by a preliminary study of vertical lobe ablation that these learning abilities may be seated in this hippocampal analogue.

Oncogenic Properties of an Epidermal Growth Factor Receptor Mutant in Glioblastoma Multiforme

Research Director: Dr. Matthew Meyerson

Glioblastoma multiforme is the most common type of malignant brain tumor in adults. Despite recent advances in genomic characterization of the disease, it presents a significant therapeutic challenge. The precise characterization of cancer causing somatic mutations has enabled effective drug development in other neoplasms. Genomic alterations in the Epidermal Growth Factor Receptor (EGFR) gene play a crucial role in the oncogenesis of glioblastoma. In this thesis, we aim to determine the functionality of a previously uncharacterized mutant, EGFRvII. The EGFRvII mutant results from deletion of exons 14-15 in the extracellular domain of EGFR. We created EGFRvII mutant cDNA using site-directed mutagenesis and stably infected Ba/F3 cells with retrovirus to assess whether EGFRvII mutant has oncogenic properties. We show that the EGFRvII mutant is able to induce cellular transformation of Ba/F3 cells in vitro. We assessed the susceptibility of Ba/F3 cells expressing EGFRvII cDNA to tyrosine kinase inhibitors and chemotherapy drugs by treating with various drug combinations. Treatment with small molecule EGFR inhibitors effectively impaired the cellular transformation by the EGFRvII mutant. This suggests patients harboring EGFRvII mutants may benefit from treatment with EGFR inhibitors, in addition to standard chemotherapy treatment.

Optogenetic control of hippocampal oscillations

Research Director: Dr. Sydney Cash

Epilepsy is a disorder that affects millions of people worldwide and is often unresponsive to current medical treatments. To better understand the origins of seizure activity in the brain we focused on the hippocampal circuitry of rats as an analog for temporal lobe epilepsy. The recent breakthrough of optogenetics allowed us an unprecedented opportunity to control specific subpopulations of neurons with high temporal specificity through light-based stimulation. Through optogenetic stimulation of the principle neurons in the hippocampus, we hoped to create and modulate hippocampal frequencies to mimic natural and pathological oscillations. We found that a lentiviral vector to express channelrhodopsin in the naive hippocampus resulted in robust channelrhodopsin expression. Also, stimulating principle neurons by laser at frequencies between 0.1 and 1 Hz was sufficient to induce ERPs in the LFP as well as increase LFP power at the stimulation frequency for the duration of stimulation. Unfortunately, higher frequency stimulation was hampered by technical oversight, but did lead to the important recognition that, at high frequencies, lasers can cause artifacts on the power spectrum of signal data. These findings led us to conclude that we did successfully drive hippocampal neurons at low frequencies, but it is not clear if that driving was identical to natural oscillations at the same frequencies.

Organization of Retinal Ganglion Cell Axons in Larval Zebrafish

Research Director: Dr. Florian Engert

A highly visual organism, the zebrafish displays many visually driven locomotor behaviors, creating an opportunity to understand the circuitry underlying these behaviors. Here, the morphological characteristics of retinal ganglion cells (RGCs), which carry signals from the eye to the brain, are investigated. First, single cell characteristics were probed, by photoconverting GFP with a two-photon laser in cell bodies of RGCs and attempting to trace single axons into retinorecipient neuropil. These single cell characteristics were also studied employing the Brainbow strategy, a system which expresses stochastic levels of fluorescent proteins in each cell, creating a multicolor array of cells. Expressing various colors in a sparse population of RGCs, the various axon types of RGCs were supposed to be labeled. However, both methods did not provide adequate fluorescent signal for any useful analyses. Photoactivatable GFP failed to diffuse at bright enough levels from the cell bodies to axonal terminal for any morphological analysis to be conducted. Titrating the right amount of fluorescent expression with the Brainbow strategy also proved unfruitful, especially due to difficulty balancing between sparse expression and brightness, resulting in unresolvable axons. Next properties of subpopulations of RGCs were investigated through fluorescent dye injection in discrete regions of the eye and tracing these subpopulations to the optic tectum and other arborization fields. These studies confirmed the retinotopic organization of RGC arbors in the optic tectum; however remain inconclusive regarding non-tectal arbors.

Role of ionic zinc in neuronal cell death and regeneration after axonal injury, The

Research Director: Dr. Larry I. Benowitz

Retinal ganglion cells (RGCs), like most other adult neuronal cells in the central nervous system (CNS), are largely unable to regenerate damaged axons through the optic nerve after injury, and instead undergo cell death. As a result, victims of traumatic brain injury or neurodegenerative diseases such as glaucoma are often left with permanent visual loss. Although recent studies have identified factors promoting cell survival and axonal regrowth, the early events that trigger the cell death pathway and inhibit regeneration after injury remain elusive. The present study aims to investigate the role of ionic zinc in RGC death and suppression of axonal regeneration after optic nerve injury by examining changes in zinc levels, time course and localization of the effect, resultant cytotoxicity, and downstream signaling pathways involved. Through in vitro studies in cell culture and in vivo studies in mice, we show that zinc levels increase dramatically as early as 1 hour after optic nerve injury, making zinc one of the earliest detectable markers of cell death. This increase is first observed in the inner plexiform layer (IPL) of the retina by means of zinc sensors and electron microscopy. The elevation in zinc concentration is toxic to RGCs, causing cell death within a few days if no treatment is introduced. However, immunohistochemical analyses reveal that zinc chelation significantly reduces RGC death and strongly promotes axonal regeneration. Taken together, these results show that zinc plays a critical role in mediating cell death and suppressing regeneration after injury, and that blocking zinc elevation enhances cell survival and axonal regeneration. These findings bring us closer to developing therapeutic treatments to induce axonal regeneration and functional recovery after nerve injury.

Role of Posttranslational Modifications in the Regulation of the Stability and Function of the Survival of Motor Neuron Protein in Response to Oxidative Stress, The

Research Director: Dr. Lee L. Rubin

Spinal muscular atrophy (SMA) is a genetic neuromuscular disorder caused by a deletion or mutation in the survival motor neuron 1 gene (SMN1), resulting in decreased levels of the Survival of Motor Neuron (SMN) protein. Although SMN is a ubiquitously expressed protein, the deficiency in SMN protein levels selectively affects the neuromuscular system and results in the degeneration of motor neurons and proximal muscle weakness. While the survival motor neuron 2 gene (SMN2) also encodes for the SMN protein, a single nucleotide difference results primarily in a truncated form of SMN (SMNΔ7), which is quickly degraded. This study investigates the role of posttranslational modifications – specifically, a phosphorylation site at Ser163 and an acetylation site at Lys 65 – in regulating SMN stability and function. To test the stability of the mutant proteins, I used a cycloheximide chase assay to measure rates of degradation by quantifying the remaining levels of SMN protein at regular time points. Preliminary results suggest that phosphorylation of Ser163 may influence SMN stability, but further experiments are necessary to determine if these results are reproducible. To study the effect of phosphorylation or acetylation on SMN function, I looked for changes in SMN localization through an image-based assay. Preliminary data suggest that modifications at Ser163 and Lys65 do not have a significant effect on SMN localization. Future studies will attempt to further elucidate the role of these posttranslational modifications on SMN function by looking at their effect on cell survival following oxidative stress.

Role of RalA and RalB in the Mammalian Hippocampus

Research Director: Dr. Thomas L. Schwarz

The process of memory formation remains one of neuroscience's most tantalizing mysteries. Advances in the field, many of them recent, have helped localize aspects of this activity to the hippocampus as well as shed light on potential neural correlates of memory acquisition. Less understood however are the molecular cues that might initiate the process and effect the physical manifestation of memory. In this thesis, I examine the role of a Ras-related protein family on dendritic spine formation and gross neuronal morphology. Using a 48-hour expression window, I analyzed the effects of RalA and RalB on transfected mammalian hippocampal cultures. Results show that RalA increases dendritic spine density and proximal neuronal branching, while RalB appears to increase spine length only. Furthermore, I show the RalA effects are mediated through a sec5-dependent pathway, suggesting a possible interaction between Ral proteins and the excoyst complex to deliver membrane-bound vesicles to growing spines and neurites.

Sensitivity of the Human Circadian Clock to Phase-Resetting Stimuli: Interactions of Melatonin, Light, and Sleep

Research Director: Dr. Elizabeth B. Klerman

Melatonin is endogenously produced and released in humans during nighttime darkness and suppressed by ocular light exposure. Exogenous melatonin is used to induce circadian phase shifts and sleep. The circadian phase-shifting ability of a stimulus (melatonin, light, or caffeine) relative to its timing can be quantified using a phase response curve (PRC). A previously developed mathematical model simulates endogenous production and clearance of melatonin as a function of circadian phase, light-induced suppression, and resetting of circadian phase by light. We extend this model first to include the pharmacokinetics of oral exogenous melatonin and phase-shifting effects via melatonin receptors in the suprachiasmatic nucleus of the mammalian hypothalamus, and then to include homeostatic modulation of the circadian phase shifting effects of exogenous melatonin. Model parameters are fit using three data sets: (i) melatonin concentration following a 0.3 or 5.0 mg dose, (ii) a PRC to a 3.0 mg dose of melatonin, and (iii) circadian phase advances following bright light stimuli in rested and sleep-deprived subjects. After fitting to the 3.0 mg PRC, the model correctly predicts that by comparison, the 0.5 mg PRC peaks both at lower amplitude and later circadian phase. This model also reproduces blood concentration profiles of various melatonin preparations that differ only in absorption rate and percentage degradation by first-pass hepatic metabolism. Finally, the model correctly predicts homeostatic modulation of circadian phase shifts in trials of simulated sleep deprivation. This is the first model of homeostatic modulation of melatonin's circadian phase shifting effects, and therefore provides new insights into the underlying physiology of the interactions between the circadian and homeostatic processes. Additionally, its ability to simulate experimental protocols using oral melatonin and sleep deprivation has application to guide dose size and timing, as well as sleep schedules, to optimally shift and entrain circadian rhythms.

Spatial memory development: a behavioral and electrophysiological study

Research Director: Dr. Charles A. Nelson

Declarative memory, memory of facts and events, is an important brain function. It has been shown to develop throughout life, with some abilities present at birth and others emerging later in childhood. Interestingly, the brain structures underlying these abilities have a parallel developmental profile. However, few studies linked this behavioral and structural development. We studied the development of spatial memory from both a behavioral and structural perspective using a Visual-Paired Comparison (VPC) task and Event-Related Potential (ERP). We assessed the performance of 9-month-olds and adults on two spatial memory tasks to test egocentric spatial memory, dependent on the maturation of the parahippocampal cortex (thought to be mature around birth), and allocentric spatial memory, dependent on the hippocampal formation (shown to develop until adulthood). Subjects learned specific locations for different objects, and were then shown each object in its familiar and a novel location simultaneously. Memory was inferred if subjects looked more at the novel object. Our preliminary data revealed a novelty preference among infants in the egocentric condition, and no preference in the allocentric condition. This supported the hypothesis that egocentric spatial memory, subserved by the parahippocampal cortex, is present by 9 months of age, but allocentric memory, subserved by the hippocampal formation, is not. This behavioral data will be complemented by electrophysiological data (ERP). This study will add to our current knowledge of declarative memory development. Understanding typical development of this function and the underlying structures will clarify neurodevelopmental disorders involving these brain regions, such as autism and Down Syndrome.

TorsinA assists in the folding and secretion of wild-type α1-antitrypsin

Research Director: Dr. Xandra O. Breakefield

TorsinA is a chaperone protein located in the lumen of the endoplasmic reticulum and the nuclear envelope. A glutamic acid deletion in the carboxyl terminus of torsinA (ΔGAG) is associated with the manifestation of early-onset dystonia (EOD), a disease characterized by sustained, involuntary muscle contractions. In this study, we used wild-type and mutant α1-antitrypsin (A1AT) as a paradigm for investigating the putative role of torsinA in the processing of proteins through the secretory pathway. HEK293 cells co-transfected with expression vectors for wild-type torsinA and A1AT resulted in elevated levels of A1AT secretion as determined by quantitative densiometric western blots and enzyme-linked immunosorbent assays (ELISA). To further distinguish the potential role of torsinA in protein folding or ER exit site regulation, we transfected HEK293 cells expressing torsinA with a vector for secreted superfolded green fluorescent protein (sfGFP), a protein processed through the secretory pathway without interactions with chaperone proteins. Levels of sfGFP secretion remained unaltered in the presence of wild-type torsinA relative to controls, suggesting a function for torsinA in protein folding rather than ER exit.

Unified Model for a Functional Algorithm Underlying Behaviors of the Cerebellum and Cerebellum-Like Structures, A

Research Director: Dr. Florian Engert

The cerebellum has been well characterized with respect to both its structure and its function. In addition to a cerebellum, elasmobranchs, the group of cartilaginous fish including sharks, skates, and rays, possess cerebellum-like structures so named for their striking structural similarity to the cerebellum; both the cerebellum and cerebellum-like structures possess a molecular layer of parallel fibers and interneurons. Cerebellum-like structures are responsible for the execution of behaviors distinct from those underlain by the cerebellum but that seem to operate based on the same functional algorithm, indicating that the type of connectivity exhibited by both structures lends itself to such an algorithm. In this thesis, I will examine two behaviors, one modulated by the cerebellum and the other by the cerebellar-like dorsal octavolateralis nucleus (DON). The vestibulo-ocular reflex (VOR) is the reflex that allows a subject to maintain focus on a single object even while moving its head. Compensation for head motion is achieved by equal and opposite motion of the eyes. If the eyes fail to perfectly oppose the head's motion, the cerebellum is responsible for modulating the behavior. The DON is responsible for the adaptive filtration of self-generated noise due to ventillatory motions in elasmobranchs. When an elasmobranch breathes, the muscle contractions involved generate weak electrical signals that are detected by the fish's own electroreceptors. In order to distinguish this self-generated signal from relevant environmental cues, the DON must adaptively subtract it from the aggregate electrical signal. I will elucidate the principles by which both behaviors operate and show that the specific type of wiring that occurs in both relevant brain areas lends itself logically and actually to error correction in both cases.

Vascular, Astrocyte, and Microglial Involvement in Cerebral Malaria

Research Director: Dr. Ryan Carroll

Cerebral malaria is an intractable neurological disease, caused by infection with the parasite Plasmodium. The present thesis was designed 1) to document parasitic sequestration in human brain vessels after infection with P. falciparum; and 2) to investigate the relationship between behavior and markers of cellular cerebral dysfunction after parasitic infection. We documented the presence of parasitic sequestration in human blood vessels by analyzing IFA and H&E stains of P. falciparum-infected sectioned human brain tissue. We found a significantly higher percentage of sequestered veins compared to arteries or capillaries, but did not find a significant difference in the percentage of parasitized vessels in any one particular brain region. To investigate the relationship between behavior and cellular dysfunction we used C57BL/6 mice infected with P. berghei strain ANKA, a model of human CM. We objectively assessed and scored mouse behavior and correlated this quantification with astrocyte and microglial activation, as evidenced by IHC analysis of the CA1 region of the hippocampus and MO region of the somatomotor cortex. We found a significantly higher microglia cell count in infected mice compared to controls in both the CA1 and MO region, as evidenced by Iba1. Furthermore, microglial count was inversely correlated with behavior score. There was no significant difference between astrocyte counts in cases and controls in either the CA1 or MO regions, as evidenced by GFAP. However, when analyzed with S100B, astrocyte counts in the MO region, but not the CA1 region, were significantly higher in cases compared to controls. The results of this thesis provide evidence for parasitic sequestration in human brain vessels and suggest a relationship between murine CM-induced microglial activation and behavior.

Visualizing the functional integration of newborn granule cells in the adult mouse olfactory bulb by long-term in vivo imaging

Research Director: Dr. Venkatesh N. Murthy

The vertebrate olfactory bulb (OB) is the primary processing center for odor information from the sensory epithelium. Interestingly, two types of OB interneurons, granule and periglomerular cells, undergo continuous turnover and replacement throughout adulthood. Progenitor cells are generated in the subventricular zone, migrate through the rostral migratory stream, and differentiate upon arrival in the OB. The need to integrate these newborn cells into a working neural system while maintaining full functionality represents a unique challenge. Efforts to examine this integration have largely focused on morphological development or in vitro neuronal population studies.

We sought to visualize the integration of individual adult-born cells functionally over extended time periods in vivo. To specifically track and measure neuronal activity from adult-born cells, we injected a lentiviral vector genetically encoding the red fluorescent protein dTomato and the green fluorescent calcium indicator GCaMP5 into the rostral migratory stream of wild-type mice. We then installed chronic cranial windows over the OB allowing us to repetitively image identified cells and dendrites over several weeks using two-photon microscopy. Using this approach, we successfully imaged odor-evoked activity of integrating adult-born cells in anesthetized mice.

We demonstrated that cells as young as approximately 10 days can show odor-evoked activity, long before they reach their morphologically complete state. Furthermore, newly integrating cells often displayed a wide odor receptive field that narrowed with time. These results support the hypothesis that adult-born cells undergo a functional critical period and that adult neurogenesis may play a role in neural circuitry plasticity of the OB.

Note: The results and conclusions stated in this abstract are still under construction at the current date of this draft. Hypothetical results inferred from data trends have been written in the last paragraph just for the sake of completing this draft. Once data has been completely analyzed, this abstract will be updated to reflect the proper conclusions.