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Cracking the bioelectric code

Probing endogenous ionic controls of pattern formation

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Patterns of resting potential in non-excitable cells of living tissue are now known to be instructive signals for pattern formation during embryogenesis, regeneration and cancer suppression. The development of molecular-level techniques for tracking ion flows and functionally manipulating the activity of ion channels and pumps has begun to reveal the mechanisms by which voltage gradients regulate cell behaviors and the assembly of complex large-scale structures. A recent paper demonstrated that a specific voltage range is necessary for demarcation of eye fields in the frog embryo. Remarkably, artificially setting other somatic cells to the eye-specific voltage range resulted in formation of eyes in aberrant locations, including tissues that are not in the normal anterior ectoderm lineage: eyes could be formed in the gut, on the tail, or in the lateral plate mesoderm. These data challenge the existing models of eye fate restriction and tissue competence maps, and suggest the presence of a bioelectric code—a mapping of physiological properties to anatomical outcomes. This Addendum summarizes the current state of knowledge in developmental bioelectricity, proposes three possible interpretations of the bioelectric code that functionally maps physiological states to anatomical outcomes, and highlights the biggest open questions in this field. We also suggest a speculative hypothesis at the intersection of cognitive science and developmental biology: that bioelectrical signaling among non-excitable cells coupled by gap junctions simulates neural network-like dynamics, and underlies the information processing functions required by complex pattern formation in vivo. Understanding and learning to control the information stored in physiological networks will have transformative implications for developmental biology, regenerative medicine and synthetic bioengineering.

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Introduction to Bioelectricity

It has long been known that all cells, not just excitable nerve and muscle, drive and respond to slow changes in transmembrane potential (V_{mem}).[1,2] Ion channels and pumps segregates charges to opposite sides of plasma and organelle membranes, producing slowly-changing differences in resting potential among cells in vivo (**Fig. 1**). Indeed spatial gradients of V_{mem} distributions exist also at the level of tissues and organs,[3] and have been long-known to correlate with important events in pattern formation such as gastrulation, neurogenesis and limb induction.[4-7] The classical data on developmental roles of endogenous bioelectric signals have recently been revitalized by the development of molecular-resolution genetic and pharmacological tools for the investigation and functional control of ionic signals in vivo.[8-10] Changes in resting potential regulate differentiation, proliferation, migration and orientation[11-13] of a wide variety of cell types, including stem cells, neurons and neuronal precursors and migratory populations such as neural crest. Recent data have implicated spatiotemporal patterns of V_{mem} in the regulation of embryonic development, regeneration and

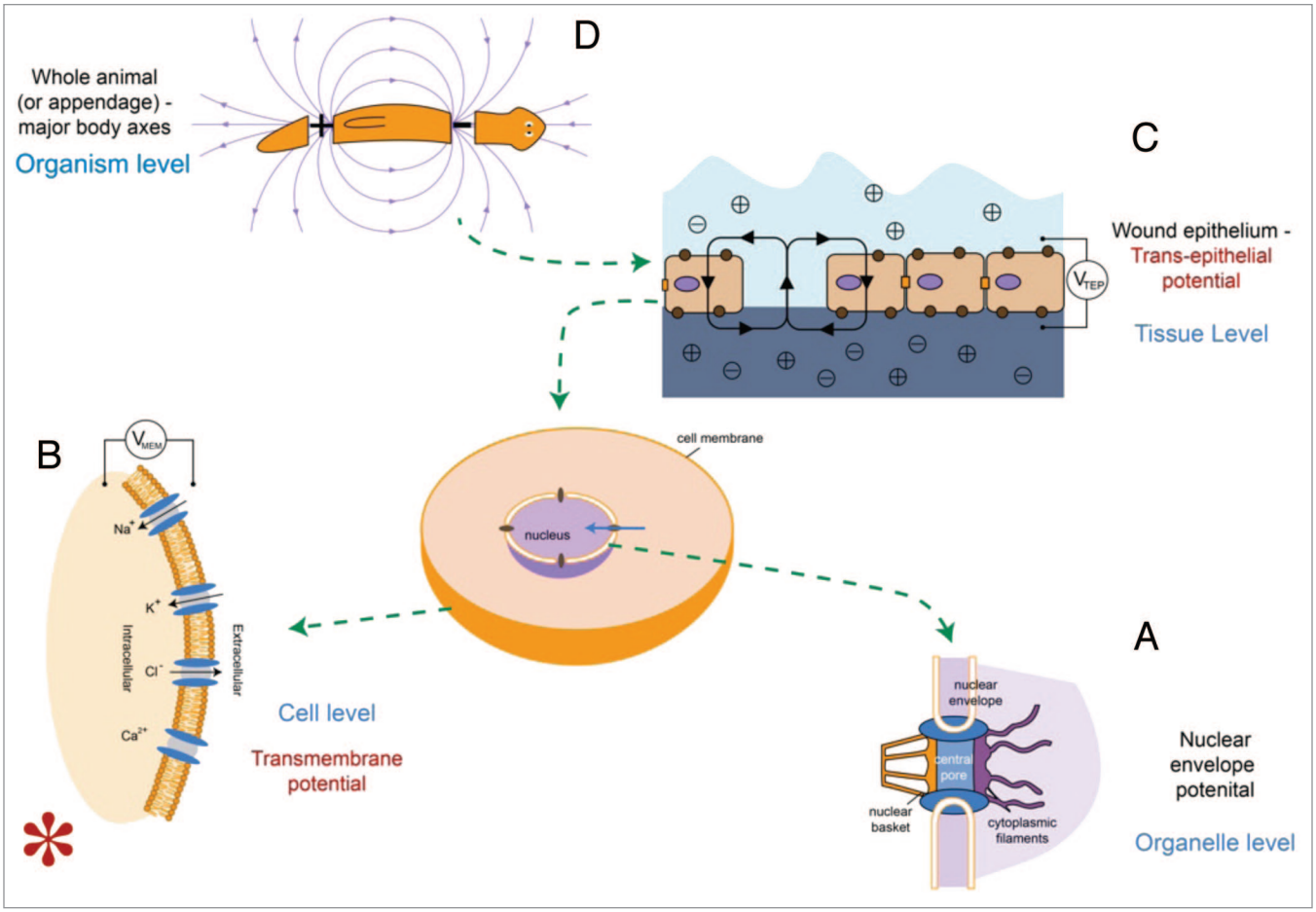

Figure 1. Multi-scale bioelectric gradients in vivo. Segregation of ions by ion channel and pump proteins, and open high-conductivity paths (through gap junctions, cytoplasm and extracellular fluids), produce gradients of voltage potential. These exist at the level of organelles (e.g., nuclear envelope potential, **A**), cells (transmembrane potential, **B**), tissues (transepithelial potential, **C**) and even whole animal axes or appendages (**D**). Schematic drawn by Maria Lobikin.

metastatic transformation.[14] Thus, bioelectric cues function alongside chemical gradients, transcriptional networks, and haptic/tensile cues as part of the morphogenetic field that orchestrates individual cell behavior into large-scale anatomical pattern formation.[15,16]

Recent work in tractable model systems has shown that bioelectric cues serve as mediators of positional information[17] and determinants of anatomical identity during growth and morphostasis.[18,19] Endogenous bioelectric fields mediate wound healing by coordinating epithelial closure,[20] initiate complex appendage regeneration,[21,22] regulate neoplastic transformation,[23,24] determining the left-right asymmetry of internal organs,[25,26] control differentiation of mammalian adult[27] and embryonic[28] stem cells, and even dictate the large-scale anatomy (producing head vs. tail) of tissues derived from the blastema in regenerating flatworms.[18] Importantly, these coherent changes of anatomy have been analyzed to reveal the mechanistic steps leading from voltage change to cell behavior,[10,29] revealing regulation of small signaling molecules' movement through transporters as a common scheme for transducing bioelectrical changes into transcriptional[23,30,31] and epigenetic[29,32,33] readouts.

Importantly, in a number of cases, it has been shown that the patterning change produced by a specific bioelectric signal (e.g., eye induction, neoplastic transformation, left-right asymmetry generation) is dependent only on the voltage itself, not on the genetic identity of the channel or the chemical species of the ion involved; in most cases, the same downstream effects on cells' behavior are induced by a given V_{mem} state regardless of whether that state was achieved by movement of potassium, sodium, or chloride ions,[19,23,26,30,34] or of which ion translocators' activity resulted in the given potential change. Because the V_{mem} of cells can be set by post-translational gating of ion channels, pumps and gap junctions (not just at the level of gene expression), this is a true epigenetic layer of control (in Waddington's original sense of the word.) The instructive signal is often borne by the physiological state itself, not the genetic identity of any particular ion channel or pump gene.

Thus, patterning information at the level of physiology regulates morphogenesis in a number of systems. In parallel with the genetic and epigenetic codes, this implies the existence of a bioelectric

code—a quantitative mapping between ionic properties of cellular structures and anatomical outcome. Further progress necessitates a synthesis—a conceptual understanding of how biophysical properties of cells may be interpreted by growing tissues into specific changes of growth and form. Interestingly, recent data suggest three different ways by which biological structures decode bioelectric patterns.

Eye Induction by Control of V_{mem}

The development of voltage-sensitive fluorescent dyes has facilitated the non-invasive tracking of ionic gradients within single cells (**Fig. 2A**) and during complex pattern formation (**Fig. 2B**).[35,36] Moreover, strategies for targeted misexpression of well-characterized ion translocator proteins allow the investigator to specifically depolarize or hyperpolarize select cell groups in vivo during loss- or gain-of-function experiments.[37] These techniques recently converged to shed new light on a paradigm case of embryonic pattern formation: eye development in the frog *Xenopus* laevis.[19]

A survey of spatiotemporal distributions of V_{mem} during craniofacial development[35] revealed two spots of hyperpolarized cells that become eyes; artificial depolarization of these cells by misexpression of a cation channel altered expression of endogenous eye markers and induced eye defects. Conversely, misexpression of a number of channels could drive non-eye-field cells into the resting potential range associated with eye induction (**Fig. 2C**), initiating a feedback loop between hyperpolarization and Pax6 expression, and causing the formation of complete eyes (**Fig. 2D**). Most excitingly, this could occur far outside the anterior neural field (where exogenous Pax6 alone cannot initiate eye formation): eyes could be formed in the gut, tail, or lateral plate mesoderm! These data significantly extend our understanding of lineage restrictions and competence during development; a specific range of resting potential was able to drive cells well outside of the anterior neurectoderm into an eye fate and form complete organs. What are the implications of these data in the wider context of the field of bioelectricity and molecular developmental biology?

Major Open Questions About the Bioelectric Code

For any code, it is crucial to ask what outcomes are mapped onto which observable properties of the coding medium. For example, the well-established genetic code maps the structure of DNA into the sequence of protein building blocks, but it does not directly specify morphology. Precisely what aspects of pattern are encoded by biophysical properties of cells? Three major (non-mutually-exclusive) possibilities have been suggested by the latest data.

The first is that of spatiotemporal gradients of V_{mem} within cells and tissues as direct prepattern for growth and form, an idea that was first proposed decades ago.[38,39] Recent studies of bioelectric changes in the developing amphibian face[35] reveal that iso-electric domains of voltage regionalize the growing tissue-bioelectric properties distributed across ell fields directly form a template controlling gene expression much as patterns of gene expression (e.g., HOX code) underlie the anatomy of vertebra, limbs and whole embryos. According to this hypothesis, the way to understand bioelectric influences is at the level of control of gene expression domains in tissue, and that this code can be capitalized upon by imposing desired patterns upon tissue by manipulation of the V_{mem} (e.g., repairing birth defects by inducing the right pattern of voltage gradients within tissue primordial that thus correct the expression patterns of key downstream patterning genes). We are currently developing optogenetic[40] strategies that would allow the real-time rewriting of bioelectric patterns in vivo.

A second possible function of the bioelectric code is a mapping of voltage ranges to specific anatomical structures. For example, a narrow range of transmembrane potential is necessary and sufficient for demarcating the eye field (**Fig. 2C and D**) while depolarized or hyperpolarized V_{mem} determines whether a head or tail forms at a wound blastema in planaria (**Fig. 2E and F**). On this model, there may be specific voltage ranges corresponding to individual organs, such as eyes, heads, or hearts (**Fig. 3A and B**); indeed, given the fact that each cell has not one but many domains of different V_{mem} along its surface (**Fig. 2A**), the spatial distribution of voltage values could form an even richer combinatorial code and store a significant amount of information for a cell as well as its neighbors. Thus, it is imperative to discover whether additional voltage ranges are associated with the formation of various organ structures, and if so, whether the quantitative nature of this mapping is the same across different model systems and across individuals of the same species. Note that this view focuses on one organizational level higher than the prepattern model, since here the functional association of specific V_{mem} values is with an entire complex organ, not with direct control of transcriptional patterns within cell fields.

Interestingly, recent data demonstrated the existence of an even higher-level effect. In the tadpole tail, during the refractory period at which the tail normally does not regenerate (**Fig. 3C and E**), induction of proton pumping or sodium influx can kick-start the regeneration of an entire appendage including spinal cord, muscle, vasculature and peripheral innervation. In both cases, very little information was provided by the initiating bioelectrical signal, as we did not modulate the ion flux in any way. These findings were most intriguing since following amputation, we could induce the regrowth of an appendage without knowing, or having to specify, the detailed structure of the organ (a finding with obvious implications for regenerative biomedicine). Instead of micromanaging its assembly (a task beyond today's bioengineering capabilities), the appropriate bioelectric signal is apparently able to activate the host's coherent, self-limiting morphogenetic subroutine corresponding to tail formation. Note that unlike the ectopic eyes and posterior heads (**Fig. 2**), in this case the appropriate structure (heart or tail) is formed at the appropriate location (**Fig. 3**) without our matching the signal to any specific property of these structures. Thus, we wondered whether in some cases, the bioelectric signal also activates a positional information pathway

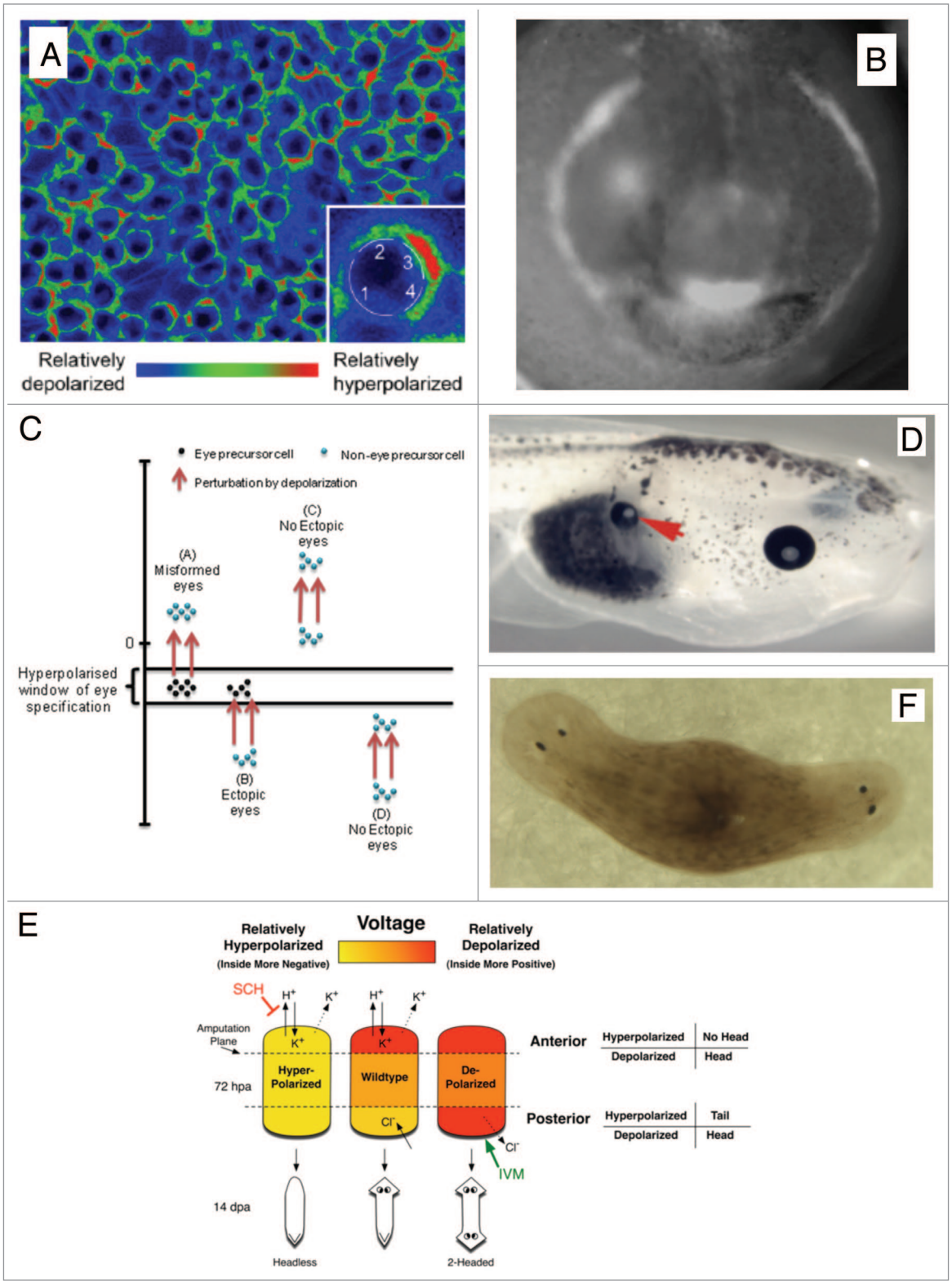

Figure 2. Bioelectric properties regulate large-scale morphology. (**A**) Voltage-sensitive dyes reveal domains within membranes of individual cells in culture—each cell contains not one but a manifold of transmembrane voltage values.[37] (**B**) At the level of organogenesis, patterns of hyperpolarized cells (lighter stain) drive craniofacial patterning in *Xenopus* laevis. These patterns determine gene expression domains (e.g., *Frizzled*) and are instructive for morphogenesis of the face.[35] Artificially setting the resting potential in embryonic frog cells in vivo to a narrow range that corresponds to eye fate (**C**) forces cell groups far outside the anterior neural field (e.g., gut or mesoderm cells) to form complete eyes (**D**).[19] Beyond single organs, manipulation of V_{mem} in accordance with a predictive model (**E**) allows rational control of large-scale pattern produced by the activity of adult stem cells in planaria; panel **F** shows a 2-headed worm in which a pharmacological technique was used to reset posterior blastema identity to that of a head by inducing the bioelectrical polarization value that determines anterior tissue fate.

that guides the induced growth toward the locally-appropriate anatomical identity.

To test this hypothesis, we used the same cocktail that induced tail regeneration (**Fig. 2E and F**) on a tadpole hindlimb amputation. We found (**Fig. 4**) no instances of a tail or any other non-limb structure forming at the wound site; instead, a number of (normally non-regenerative) animals grew back hindlimbs, including the most distal components (toes and even toe-nails); these limbs were sensitive to touch and motile (see Video S1). This remarkable effect is not only promising with respect to biomedical use of this technology to induce repair and regeneration of complex structures, but also reveals that the same biophysical signal can induce different (appropriate) structures at distinct anatomical locations.

Conclusion and Hypothesis: What's Next for Molecular Bioelectricity?

The field of bioelectricity is at an extremely exciting juncture. Molecular-level tools now exist, and a number of recent papers have demonstrated the importance of these physiological signals and shown how they interface to canonical biochemical/genetic pathways.[9,10,13] These data have taught us a number of important lessons. First, that resting potential is not simply a basic parameter needed for cell health. Much as information is routinely encoded in a subtle modulatory signal coexisting with a strong carrier wave in radio-communications, it is possible to experimentally dissociate the bioelectricity needed for housekeeping processes from the developmental roles of voltage patterns. Second, experimental modulation of voltage in vivo is a powerful method for making large-scale, coherent changes in anatomy. Thus, patterns of ionic properties among non-excitable cells are endogenous instructive parameters that not only control basic behaviors of individual cells, but also serve as regulators of shape at the organ and whole organism level. As such, these pathways are a tractable and attractive control point for biomedical strategies in the areas of birth defects, regeneration and cancer suppression.

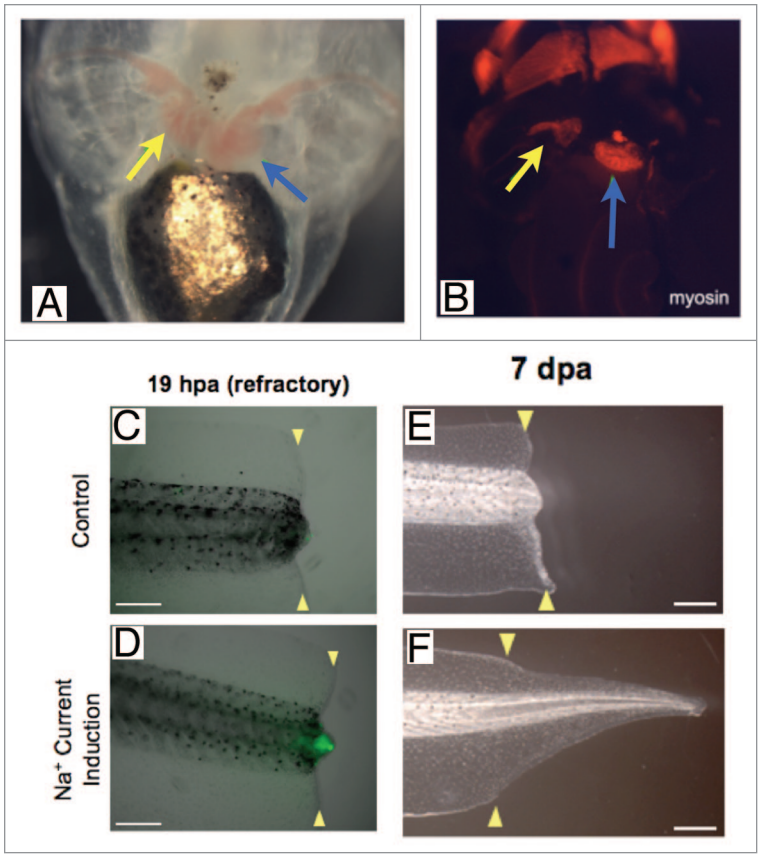

Figure 3. Bioelectric signals produce appropriate organs at appropriate locations. (**A and B**) Misexpression of specific ion channels (unpublished data, obtained by Sherry S. Aw) induces beating, ectopic hearts (indicated by yellow arrows) to be formed next to the original (primary heart, indicated by blue arrows). Likewise, at a stage when tails no longer regenerate in tadpoles (**C and E**) simple induction of a 1 h sodium flux into the tail (**D**) causes regeneration (**E**) of the entire tail.[21] In both cases, the information content of the bioelectric event is very low—a simple signal can kickstart a complex, downstream morphogenetic cascade appropriate to the location within the host.

The next steps in this field[41] must involve expanded efforts to obtain comprehensive physiomic profiling data that can be mined for quantitative analysis of the bioelectric code. Since many different ion channels can contribute to the same V_{mem}, and many different V_{mem} levels can arise from the post-translational gating of exactly the same set of channels and pumps, proteomic or transcriptome analysis inevitably miss information inherent in bioelectric properties. The profiling data, using combinations of electrodes and reporter dyes, must be merged with functional analyses using state-of-the-art methods for manipulating V_{mem} in vivo. One of the most exciting future lines of research concerns the development of chemical strategies for conferring light sensitivity to native ion channels,[42,43] allowing optical control of ion flux with heretofore unprecedented spatiotemporal resolution. As these pathways become better understood, bioelectric elements will be encapsulated as modules that can be plugged into existing bioengineering frameworks, adding greatly to the power of the current set of building blocks in synthetic biology.[44,45]

Looking further ahead beyond the immediate biomedical and engineering applications of bioelectricity, fully unlocking the promise of bioelectricity will require a novel conceptual apparatus with which to understand and learnt to exploit the dynamics of information encoded in the real-time dynamics of physiological (electrical) networks among tissues (**Fig. 5A**). Bioinformatics and modeling tools must be developed

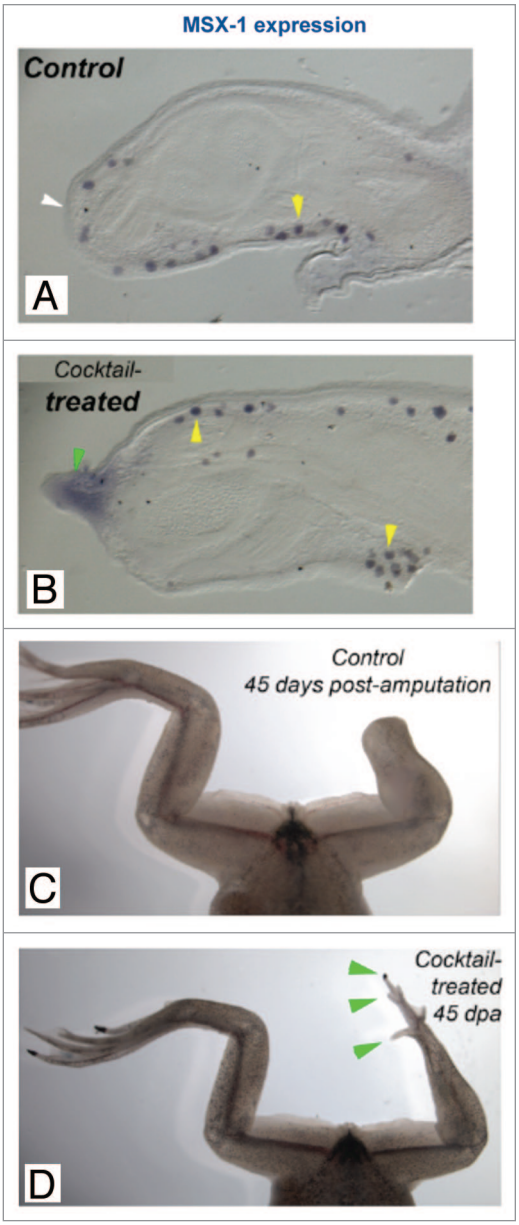

Figure 4. Na^+ induction promotes limb regeneration. To determine whether the sodium influx that triggered tail regeneration was specific for tail identity or triggered events that interacted with positional information cues (initiated a spatially-appropriate response), we tested the same monensin cocktail that initiated tail regeneration on a tadpole leg amputation model. Hind limbs of st. 57 tadpoles were amputated at the tibia-fibula location. At 1 dpa, tapoles were treated either with 0.1% Ethanol (Control) or with a cocktail of 20uM Monensin and 90mM Na-Gluconate in 0.1XMMR for 1 h. Tadpoles were allowed to recover and grown at 23C until froglet stage. Sectioning during early stages revealed that compared with control limbs (**A**), cocktail-treated limbs upregulated MSX-1, a regenerative marker at the wound edge [green arrowhead, compare with lack of MSX-1-positive region in (**A**) indicated by white arrowhead (**B**)]. Yellow arrowheads indicate additional cells expressing MSX-1 away from the wound (present in all limbs, treated or controls). Within 45 d, compared with very little or no (**C**) growth in controls, relatively improved regrowth at the amputation site was seen in the treated animals (**D**). These regenerated hindlimbs often included toes and toenails (green arrowheads), indicative of proper distal morphogenesis. Notably, in no cases (n = 28) were non-limb structures observed.

that truly capture the unique signaling properties of physiological networks and merge them with the current crop of techniques that focus on gene-regulatory networks in multi-scale models of complex biological systems. The existing models of cardiac and brain physiology are not directly suitable because they focus on action potentials and spiking dynamics, rather than the very different propagation and slow changes of resting potential that occur in non-excitable tissues.

However, we speculate that the tools of cognitive neuroscience can be extended to understand the electrical communication among non-neuronal cells. During regeneration and embryogenesis, organs frequently determine their size, ascertain their shape relative to a target morphology, recognize specific stimuli, exhibit habituation and sensitization to signals, and make decisions based on current information and data stored from prior events. We now know that many of these processes are under control of bioelectrical mechanisms. Is it possible that the ability of organs and tissues to remodel and dynamically maintain/restore specific 3-dimensional shape is a result of computations they perform via electric signaling[46]? The cognitive properties of neural networks, and the success of information processing technologies (e.g., computer science) strongly suggest that bioelectrical communication is a universal and convenient medium by which to control complex events like pattern formation. Although this remains to be tested, such a hypothesis is highly compatible with evolutionary conservation of fundamental mechanisms and the increasingly-observed parallels between developmental mechanisms and neural information processing.[47]

As is beginning to be appreciated for astrocyte and glial networks,[48,49] and has been suggested for bone[50] and even plant cells,[51] many different interconnected cell types could be functionally isomorphic with neural networks (**Fig. 5B**). Resting V_{mem} levels are analogous to the "node activation" of neural net models, while voltage-sensitive, often asymmetric gap junctions mediating cell:cell links can readily play the role of synapses. Thus, our hypothesis is that the existing highly successful theoretical apparatus for information processing in neurobiology could be extended to understand the properties of highly dynamic, self-repairing tissues and organs.[15,52] We are embarking on a

research program to determine whether quantitative, predictive models of information storage and exchange can be used to explain the cognitive-like functions of morphogenetic systems. This is an interdisciplinary effort that blurs the line between the mechanisms of spatial information (shape, target morphology) and those of temporal information (pattern in time-dependent signals, learning and memory). We hypothesize that the rich and deep techniques of computational neuroscience can be applied to understand and manipulate the functions of dynamically remodeling tissues. If true, such unification would result in truly transformative advances in synthetic morphology, bioengineering, hybrid cybernetic biorobotics and regenerative medicine.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplemental Material

Supplemental material may be found here: www.landesbioscience.com/journals/cib/article/22595

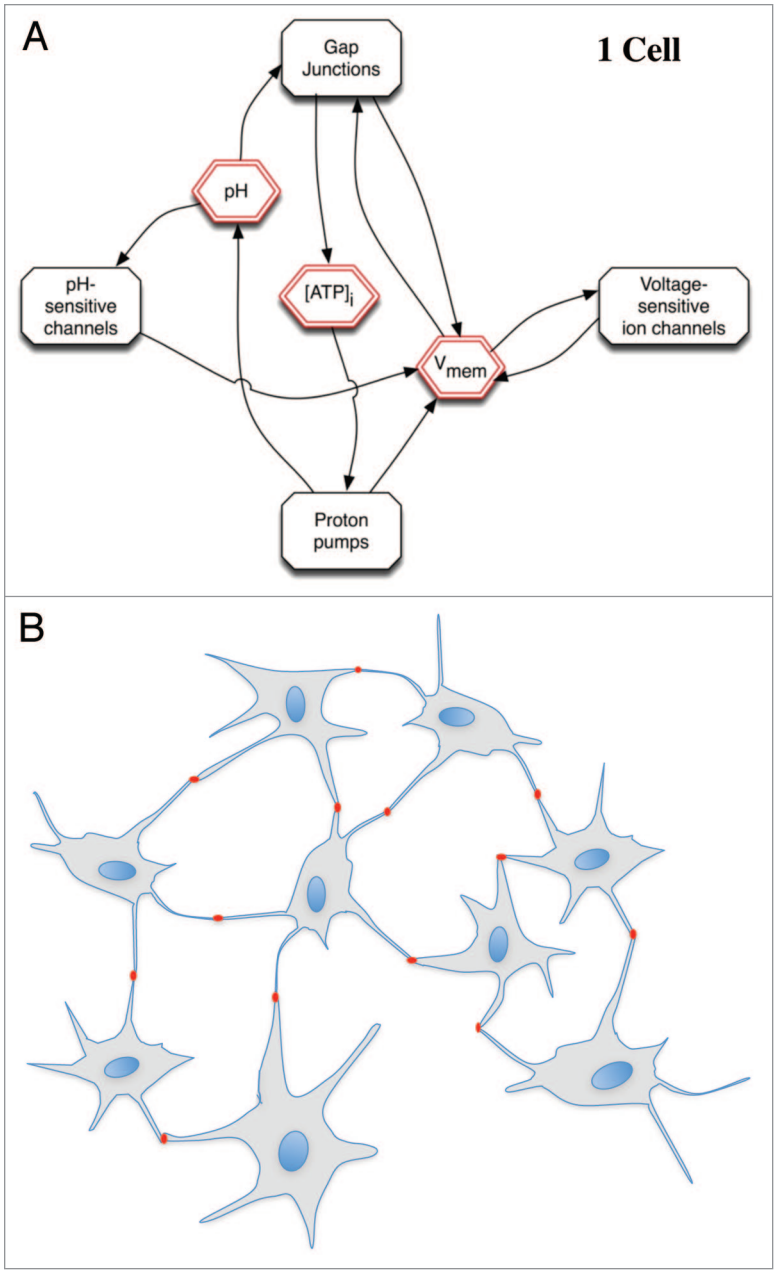

Figure 5. Physiological networks can store information. (**A**) At the level of a single cell, ion translocator proteins are both regulated by, and regulate, voltage. The network of physiological parameters and post-translational states of ion channels and pumps implements rich feedback loops, which can support multistable attractor states and thus store information in the resting potential state of cells.[53] (**B**) Collections of cells coupled by gap junctions implement neural-like networks, since the voltage-sensitive gap junctions play the role of synapses, and each cell has the ability to occupy multiple V_{mem} states based on electrical signals from its neighbors. It is thus very likely that computational tissues could be made from non-excitable cells.

References

1. Lund E. Bioelectric fields and growth. Austin: Univ. of Texas Press, 1947.
2. Levin M, Stevenson CG. Regulation of cell behavior and tissue patterning by bioelectrical signals: challenges and opportunities for biomedical engineering. Annu Rev Biomed Eng 2012; 14:295-323; PMID:22809139; http://dx.doi.org/10.1146/annurev-bioeng-071811-150114.
3. Robinson K, Messerli M. Electric embryos: the embryonic epithelium as a generator of developmental information. In: McCaig C, ed. Nerve Growth and Guidance. Portland: Portland Press, 1996:131-41.
4. Nuccitelli R, Robinson K, Jaffe L. On electrical currents in development. Bioessays 1986; 5:292-4; PMID:2436612; http://dx.doi.org/10.1002/bies.950050616.
5. Nuccitelli R. Endogenous ionic currents and DC electric fields in multicellular animal tissues. Bioelectromagnetics 1992; (Suppl 1): 147-57; PMID:1285710; http://dx.doi.org/10.1002/bem.2250130714.
6. Robinson KR, Messerli MA. Left/right, up/down: the role of endogenous electrical fields as directional signals in development, repair and invasion. Bioessays 2003; 25:759-66; PMID:12879446; http://dx.doi.org/10.1002/bies.10307.
7. Jaffe LF. Control of development by ionic currents. In: Cone RA, and John E. Dowling, eds., ed. Membrane Transduction Mechanisms. New York: Raven Press, 1979.

8. Reid B, Zhao M. Ion-selective self-referencing probes for measuring specific ion flux. Commun Integr Biol 2011; 4:524-7; PMID:22046453.
9. Levin M. Bioelectric mechanisms in regeneration: Unique aspects and future perspectives. Semin Cell Dev Biol 2009; 20:543-56; PMID:19406249; http://dx.doi.org/10.1016/j.semcdb.2009.04.013.
10. Levin M. Large-scale biophysics: ion flows and regeneration. Trends Cell Biol 2007; 17:261-70; PMID:17498955; http://dx.doi.org/10.1016/j.tcb.2007.04.007.
11. Blackiston DJ, McLaughlin KA, Levin M. Bioelectric controls of cell proliferation: ion channels, membrane voltage and the cell cycle. Cell Cycle 2009; 8:3519-28; PMID:19823012; http://dx.doi.org/10.4161/cc.8.21.9888.
12. Yao L, McCaig CD, Zhao M. Electrical signals polarize neuronal organelles, direct neuron migration, and orient cell division. Hippocampus 2009; 19:855-68; PMID:19280605; http://dx.doi.org/10.1002/hipo.20569.
13. McCaig CD, Song B, Rajnicek AM. Electrical dimensions in cell science. J Cell Sci 2009; 122:4267-76; PMID:19923270; http://dx.doi.org/10.1242/jcs.023564.
14. Levin M. Molecular bioelectricity in developmental biology: new tools and recent discoveries: control of cell behavior and pattern formation by transmembrane potential gradients. Bioessays 2012; 34:205-17; PMID:22237730; http://dx.doi.org/10.1002/bies.201100136.
15. Levin M. Morphogenetic fields in embryogenesis, regeneration, and cancer: non-local control of complex patterning. Biosystems 2012; 109:243-61; PMID:22542702; http://dx.doi.org/10.1016/j.biosystems.2012.04.005.
16. Burr HS, Northrop FSC. The electro-dynamic theory of life. Q Rev Biol 1935; 10:322-33; http://dx.doi.org/10.1086/394488.
17. Shi R, Borgens RB. Three-dimensional gradients of voltage during development of the nervous system as invisible coordinates for the establishment of embryonic pattern. Dev Dyn 1995; 202:101-14; PMID:7734729; http://dx.doi.org/10.1002/aja.1002020202.
18. Beane WS, Morokuma J, Adams DS, Levin M. A chemical genetics approach reveals H,K-ATPase-mediated membrane voltage is required for planarian head regeneration. Chem Biol 2011; 18:77-89; PMID:21276941; http://dx.doi.org/10.1016/j.chembiol.2010.11.012.
19. Pai VP, Aw S, Shomrat T, Lemire JM, Levin M. Transmembrane voltage potential controls embryonic eye patterning in Xenopus laevis. Development 2012; 139:313-23; PMID:22159581; http://dx.doi.org/10.1242/dev.073759.
20. Zhao M, Song B, Pu J, Wada T, Reid B, Tai G, et al. Electrical signals control wound healing through phosphatidylinositol-3-OH kinase-gamma and PTEN. Nature 2006; 442:457-60; PMID:16871217; http://dx.doi.org/10.1038/nature04925.
21. Tseng AS, Beane WS, Lemire JM, Masi A, Levin M. Induction of vertebrate regeneration by a transient sodium current. J Neurosci 2010; 30:13192-200; PMID:20881138; http://dx.doi.org/10.1523/JNEUROSCI.3315-10.2010.
22. Adams DS, Masi A, Levin MH. H+ pump-dependent changes in membrane voltage are an early mechanism necessary and sufficient to induce Xenopus tail regeneration. Development 2007; 134:1323-35; PMID:17329365; http://dx.doi.org/10.1242/dev.02812.
23. Blackiston D, Adams DS, Lemire JM, Lobikin M, Levin M. Transmembrane potential of GlyCl-expressing instructor cells induces a neoplastic-like conversion of melanocytes via a serotonergic pathway. Dis Model Mech 2011; 4:67-85; PMID:20959630; http://dx.doi.org/10.1242/dmm.005561.
24. Morokuma J, Blackiston D, Adams DS, Seebohm G, Trimmer B, Levin M. Modulation of potassium channel function confers a hyperproliferative invasive phenotype on embryonic stem cells. Proc Natl Acad Sci USA 2008; 105:16608-13; PMID:18931301; http://dx.doi.org/10.1073/pnas.0808328105.
25. Levin M. Is the early left-right axis like a plant, a kidney, or a neuron? The integration of physiological signals in embryonic asymmetry. Birth Defects Res C Embryo Today 2006; 78:191-223; PMID:17061264; http://dx.doi.org/10.1002/bdrc.20078.
26. Levin M, Thorlin T, Robinson KR, Nogi T, Mercola M. Asymmetries in H+/K+-ATPase and cell membrane potentials comprise a very early step in left-right patterning. Cell 2002; 111:77-89; PMID:12372302; http://dx.doi.org/10.1016/S0092-8674(02)00939-X.
27. Sundelacruz S, Levin M, Kaplan DL. Membrane potential controls adipogenic and osteogenic differentiation of mesenchymal stem cells. PLoS ONE 2008; 3:e3737; PMID:19011685; http://dx.doi.org/10.1371/journal.pone.0003737.
28. Lange C, Prenninger S, Knuckles P, Taylor V, Levin M, Calegari F. The H(+) vacuolar ATPase maintains neural stem cells in the developing mouse cortex. Stem Cells Dev 2011; 20:843-50; PMID:21126173; http://dx.doi.org/10.1089/scd.2010.0484.
29. Tseng A-S, Levin M. Transducing bioelectric signals into epigenetic pathways during tadpole tail regeneration. Anat Rec 2012; In press; http://dx.doi.org/10.1002/ar.22495.
30. Adams DS, Robinson KR, Fukumoto T, Yuan S, Albertson RC, Yelick P, et al. Early, H+-V-ATPase-dependent proton flux is necessary for consistent left-right patterning of non-mammalian vertebrates. Development 2006; 133:1657-71; PMID:16554361; http://dx.doi.org/10.1242/dev.02341.
31. Fukumoto T, Kema IP, Levin M. Serotonin signaling is a very early step in patterning of the left-right axis in chick and frog embryos. Curr Biol 2005; 15:794-803; PMID:15886096; http://dx.doi.org/10.1016/j.cub.2005.03.044.
32. Tseng AS, Carneiro K, Lemire JM, Levin M. HDAC activity is required during Xenopus tail regeneration. PLoS ONE 2011; 6:e26382; PMID:22022609; http://dx.doi.org/10.1371/journal.pone.0026382.
33. Carneiro K, Donnet C, Rejtar T, Karger BL, Barisone GA, Díaz E, et al. Histone deacetylase activity is necessary for left-right patterning during vertebrate development. BMC Dev Biol 2011; 11:29; PMID:21599922; http://dx.doi.org/10.1186/1471-213X-11-29.
34. Aw S, Adams DS, Qiu D, Levin MH. H,K-ATPase protein localization and Kir4.1 function reveal concordance of three axes during early determination of left-right asymmetry. Mech Dev 2008; 125:353-72; PMID:18160269; http://dx.doi.org/10.1016/j.mod.2007.10.011.
35. Vandenberg LN, Morrie RD, Adams DS. V-ATPase-dependent ectodermal voltage and pH regionalization are required for craniofacial morphogenesis. Dev Dyn 2011; 240:1889-904; PMID:21761475; http://dx.doi.org/10.1002/dvdy.22685.
36. Adams DS, Levin M. General principles for measuring resting membrane potential and ion concentration using fluorescent bioelectricity reporters. Cold Spring Harb Protoc 2012; 2012:385-97; PMID:22474653; http://dx.doi.org/10.1101/pdb.top067710.
37. Adams DS, Levin M. Endogenous voltage gradients as mediators of cell-cell communication: strategies for investigating bioelectrical signals during pattern formation. Cell Tissue Res 2012; PMID:22350846; http://dx.doi.org/10.1007/s00441-012-1329-4.
38. Burr HS, Hovland CI. Bio-Electric Correlates of Development in Amblystoma. Yale J Biol Med 1937; 9:540-9; PMID:21433741.
39. Burr HS, Northrop FSC. Evidence for the Existence of an Electro-Dynamic Field in Living Organisms. Proc Natl Acad Sci USA 1939; 25:284-8; PMID:16577899; http://dx.doi.org/10.1073/pnas.25.6.284.
40. Fenno L, Yizhar O, Deisseroth K. The development and application of optogenetics. Annu Rev Neurosci 2011; 34:389-412; PMID:21692661; http://dx.doi.org/10.1146/annurev-neuro-061010-113817.
41. Levin M. The wisdom of the body: future techniques and approaches to morphogenetic fields in regenerative medicine, developmental biology and cancer. Regen Med 2011; 6:667-73; PMID:22050517; http://dx.doi.org/10.2217/rme.11.69.
42. Fortin DL, Dunn TW, Kramer RH. Engineering light-regulated ion channels. Cold Spring Harb Protoc 2011; 2011:579-85; PMID:21632787; http://dx.doi.org/10.1101/pdb.top112.
43. Fortin DL, Dunn TW, Fedorchak A, Allen D, Montpetit R, Banghart MR, et al. Optogenetic photochemical control of designer K+ channels in mammalian neurons. J Neurophysiol 2011; 106:488-96; PMID:21525363; http://dx.doi.org/10.1152/jn.00251.2011.
44. Regot S, Macia J, Conde N, Furukawa K, Kjellén J, Peeters T, et al. Distributed biological computation with multicellular engineered networks. Nature 2011; 469:207-11; PMID:21150900; http://dx.doi.org/10.1038/nature09679.
45. Bashor CJ, Horwitz AA, Peisajovich SG, Lim WA. Rewiring cells: synthetic biology as a tool to interrogate the organizational principles of living systems. Annu Rev Biophys 2010; 39:515-37; PMID:20192780; http://dx.doi.org/10.1146/annurev.biophys.050708.133652.
46. Hauser H, Ijspeert AJ, Füchslin RM, Pfeifer R, Maass W. Towards a theoretical foundation for morphological computation with compliant bodies. Biol Cybern 2012; PMID:22290137.
47. Boettiger A, Ermentrout B, Oster G. The neural origins of shell structure and pattern in aquatic mollusks. Proc Natl Acad Sci USA 2009; 106:6837-42; PMID:19351900; http://dx.doi.org/10.1073/pnas.0810311106.
48. Blomstrand F, Aberg ND, Eriksson PS, Hansson E, Rönnbäck L. Extent of intercellular calcium wave propagation is related to gap junction permeability and level of connexin-43 expression in astrocytes in primary cultures from four brain regions. Neuroscience 1999; 92:255-65; PMID:10392848; http://dx.doi.org/10.1016/S0306-4522(98)00738-6.
49. Pannasch U, Vargová L, Reingruber J, Ezan P, Holcman D, Giaume C, et al. Astroglial networks scale synaptic activity and plasticity. Proc Natl Acad Sci USA 2011; 108:8467-72; PMID:21536893; http://dx.doi.org/10.1073/pnas.1016650108.
50. Turner CH, Robling AG, Duncan RL, Burr DB. Do bone cells behave like a neuronal network? Calcif Tissue Int 2002; 70:435-42; PMID:12149636; http://dx.doi.org/10.1007/s00223-001-1024-z.
51. Volkov AG, Carrell H, Adesina T, Markin VS, Jovanov E. Plant electrical memory. Plant Signal Behav 2008; 3:490-2; PMID:19704496; http://dx.doi.org/10.4161/psb.3.7.5684.
52. Vandenberg LN, Adams DS, Levin M. Normalized shape and location of perturbed craniofacial structures in the Xenopus tadpole reveal an innate ability to achieve correct morphology. Dev Dyn 2012; 241:863-78; PMID:22411736; http://dx.doi.org/10.1002/dvdy.23770.
53. Gallaher J, Bier M, van Heukelom JS. First order phase transition and hysteresis in a cell's maintenance of the membrane potential--An essential role for the inward potassium rectifiers. Biosystems 2010; 101:149-55; PMID:20566338; http://dx.doi.org/10.1016/j.biosystems.2010.05.007.