Morphological coordination:
A common ancestral function unifying neural and non-neural signaling

Chris Fields,[1] Johanna Bischof[2] and Michael Levin[3,*]

[1]23 Rue des Lavandières, 11160 Caunes Minervois, France, fieldsres@gmail.com
(ORCID: 0000-0002-4812-0744)

[2]Allen Discovery Center at Tufts University, Medford, MA 02155 USA, johanna.bischof@tufts.edu
(ORCID: 0000-0002-5668-841X)

[3]Allen Discovery Center at Tufts University, Medford, MA 02155 USA, michael.levin@tufts.edu
(ORCID: 0000-0001-7292-8084)

*Author for correspondence: michael.levin@tufts.edu

Abstract

Nervous systems are traditionally thought of as providing sensing and behavioral coordination functions at the level of the whole organism. What is the evolutionary origin of the mechanisms enabling the nervous systems' information processing ability? Here we review evidence from evolutionary, developmental, and regenerative biology suggesting a deeper, ancestral function of both pre-neural and neural cell-cell communication systems: the long-distance coordination of cell division and differentiation required to create and maintain body-axis symmetries. This conceptualization of the function of nervous-system activity sheds new light on the evolutionary transition from the morphologically rudimentary, non-neural Porifera and Placazoa to the complex morphologies of Ctenophores, Cnidarians and Bilaterians. It further allows a sharp formulation of the distinction between long-distance axis-symmetry coordination based on external coordinates, e.g. by whole-organism scale trophisms as employed by plants and sessile animals, and coordination based on body-centered coordinates as employed by motile animals. Thus, we suggest that the systems that control animal behavior evolved from ancient mechanisms adapting pre-existing ionic and neurotransmitter mechanisms to regulate individual cell behaviors during morphogenesis. An appreciation of the ancient, non-neural origins of bioelectrically-mediated computation suggests new approaches to the study of embryological development, including embryological dysregulation, cancer, regenerative medicine, and synthetic bioengineering.

Keywords: evolution, multicellularity, bioelectricity, neural, development

Running title: Ancestral functions of bioelectric circuits

Introduction

A critical aspect of physiology is the regulation of body functions by the nervous system, at all levels from cells to the entire organism. Outside of this traditionally-considered function, neural signaling is increasingly recognized to also be important for embryonic patterning (Herrera-Rincon et al., 2017), cancer (Pawlowski and Weddell, 1967), and regeneration (Kumar and Brockes, 2012). Importantly, neurons utilize ancient mechanisms such as ion channels, electrical synapses, and neurotransmitters that also operate throughout the body in many non-excitable tissues and predate the evolution of specialized neurons. We here propose a model in which both neuronal signals and non-neural bioelectric patterning signals arise from modifications of conserved basic machinery, and co-evolved to function to control both organismal behavior and development. Thus, understanding the relationship between brain and body, and exploiting the versatile signaling capabilities of the nervous system for biomedical purposes, requires an appreciation of the origin and adaptive functions of neurons. Here, we review the current state of the art, controversies, and knowledge gaps concerning the evolutionary history of bioelectric signaling across development, physiology, and behavior and offer a more inclusive view of nervous system function in these different contexts.

The earliest detectable event in the Metazoan lineage is the divergence, roughly 700 million years ago (Mya), between the morphologically rudimentary, aneural Porifera (sponges) and Placozoa, and the Eumetazoa (Ctenophores, Cnidarians and Bilaterians) (Erwin, 2015; Sebé-Pedrós, Degnan, and Ruiz-Rillo, 2017; see Dohrmann and Wörheide, 2017 for evidence supporting earlier dates). All Eumetazoans exhibit complex,

multicellular, and invariant morphologies characterized by multiple differentiated cell types, symmetries and specific asymmetries around well-defined body axes, and specialized nervous systems (Feuda et al., 2017; Simion et al, 2017; see Whelen et al., 2017 and below for the alternative hypothesis that Ctenophores represent the initial divergence and neither complex morphologies nor nervous systems are synaptomorphic). What explains the correlation, on both sides of this divergence, between complex, multicellular animal morphologies and nervous systems? Is there something about the morphologies, not just the behavior, of animals that demands the resource-intensive construction and maintenance of a nervous system? Why are brains (Herrera-Rincon and Levin, 2018; Herrera-Rincon et al., 2017) and spinal cord structures (Mondia et al., 2011) apparently required for normal development and regeneration (Kumar and Brockes, 2012) in morphologically complex metazoans?

Nervous systems are traditionally thought of as enabling sensing and behavioral coordination functions at the level of the whole animal (Arendt et al., 2015; Arendt, Tosches and Marlow, 2016; de Wiljes et al., 2015; de Wiljes et al., 2017; Jékely et al., 2015; Keijzer, 2015; Keijzer and Arnellos, 2017; Keijzer et al., 2013; Nielsen, 2008). From this perspective, nervous systems make complex morphologies *useful*; this increase in utility confers selective advantages that offset the resource costs of building and maintaining a nervous system. Here we review evidence from evolutionary, developmental, and regenerative biology suggesting that nervous systems also function to enable the precise, long-distance coordination of cell proliferation and differentiation that is required to create and maintain a body comprising multiple distinct cell types organized into specialized structures including organs and limbs. If this hypothesis is correct, nervous systems make complex morphologies *possible*. The competitive advantages they confer are the competitive advantages of morphological complexity itself.

The first multicellular fauna for which direct fossil evidence is available, the Ediacaran, is already morphologically and by inference behaviorally complex (Narbonne, Xiao and Shields, 2012; Darroch et al., 2018); hence the relative evolutionary timing of morphological and behavioral complexity remains a matter of hypothesis. The “skin-brain” hypothesis that nervous systems evolved to coordinate the behavior of contractile epithelia (Keijzer, van Duijn and Lyon, 2013) supposes a gradualist co-evolution of behavior and morphology supported by a gradual elaboration of a nervous system primarily dedicated to coordination of initially-simple bodily movements, e.g. body-tube contraction (de Wiljes et al., 2015; de Wiljes et al., 2017; Jékely et al., 2015; Keijzer, 2015; Keijzer and Arnellos, 2017). Recent modeling results motivated by the hypothesis that all organisms must minimize Bayesian surprise (Friston, 2013; Friston et al., 2015; Kuchling et al., 2019) suggest, however, that multicellular bodies may have evolved independently of motile capability to protect dividing cells from a hostile environment (Fields and Levin, 2019). The primary functions of cell-cell communication in such bodies would have been the suppression by dividing cells of daughter-cell proliferation and the induction of daughter-cell terminal differentiation, functions that elongated cells could perform at greater than typical nearest-neighbor distances. The selective advantage conferred by elongated cells specialized for communication – protoneurons – in this scenario is not motility, but protection of the dividing (i.e. proto-stem) cell population from the environment. Such signaling cells would, however, be available for co-option to coordinate motility. The sensory and behavioral coordination functions of nervous systems can, in this latter scenario, be interpreted as exaptations initially, and as co-evolving adaptations following functional co-option. The traits gained by having a nervous system designed for morphological control can, in this case, be co-opted for other tasks that become possible through complex morphologies, such as macrophagy and its associated complex behaviors.

If the original, ancestral function of the nervous system is the precise, long-distance (relative to typical nearest neighbor distances) coordination of cell division and differentiation, one would expect the morphology of the nervous system to reflect this function. Effective coordination of cellular behavior across a multicellular body is an example of feedback-driven control: the current control signals depend, via a feedback signal, on the previous state of the structures or functions being controlled (Ashby, 1956). In general control theory, the Good Regulator theorem (Conant and Ashby, 1970) states that any effective controller of a system must incorporate a model of that system. One could expect, therefore, the morphology of the nervous system to implement a model of the morphology of the body. The well-known somatosensory and motor "homunculi" of the human brain (Penfield and Boldrey, 1937) are examples of such models; however, the criteria for determining that a structure or function serves as a "model" of another structure or function are not well-defined outside of engineering, and may be expected, in general, to depend on larger-scale boundary conditions or context-setting parameters (e.g. Pattee, 2001; Polanyi, 1968; Rosen, 1986). For a structure or process to be a model of some other structure or process, in particular, it must be *used as a model* (set-point, or stop condition) in the context of feedback regulation, as the somatosensory and motor homunculi in the brain are used. The complexity of the morphological model can be expected to increase rapidly as the number of long-distance constraints on the relative sizes and shapes of structures built on different body axes increases. Encephalization, in this case, can be viewed as an adaptive response to the challenge of successful morphogenesis: it centralizes the morphological model and hence centralizes the enforcement of constraints that are dependent on information from distal parts of the body and therefore cannot be enforced using purely locally-sourced information.

In facultative multicellular systems such as *Dictyostelium*, small-molecule morphogens induce cell-cycle arrest and differentiation in cells that adopt

supportive and/or protective – i.e. somatic – morphologies and functions (Morris et al., 1987; 1988). In morphologically-complex unicellular organisms such as *Micrasterias*, spatially-organized bioelectric currents direct morphological differentiation following cell division (Meindl, 1993; Lutz-Meindl, 2016). Both morphogen and non-neural bioelectric signaling also function to control cell proliferation and differentiation throughout development in the Eumetazoa (Tseng and Levin, 2013; Levin, 2014; Pezzulo and Levin, 2015; 2016; Fields and Levin, 2018). Reconceptualizing the function of nervous systems in terms of morphological coordination renders signaling in nervous systems functionally continuous with these more ancient mechanisms. Nervous systems become, in particular, a novel means of extending both the range and targeting precision of these earlier systems, as they allow information generated in one part of the body to be used to control, with high spatial and temporal resolution, cellular processes in another, distal part of the body. They enable signal-generating cells to sense and control the proliferation and differentiation not just of their near neighbors, but of precise populations of distal cells anywhere in the body.

One way to conceptualize bioelectrical systems in general (both neural and non-neural) is as controllers that manage the trajectory of a complex system through state space. In development, bioelectrical networks made up of pre-neural cells guide the movement of the body through a virtual morphospace – the various anatomical configurations of embryogenesis (Stone, 1997; Gerber, 2017) (Figure 1B). This operates during regeneration and remodeling as well, where cell activity must be coordinated toward the repair of complex structures (Vandenberg, Adams and Levin, 2012; Sullivan, Emmons-Bell and Levin, 2016). However, this ancient property of control systems required for metazoan morphogenesis could have been readily coopted to manage the trajectory of the body through 3-dimensional space during animal behavior. This is exemplified both by non-neural bioelectric control of contraction in glass sponges (Leys, 2015) (Figure 1C), as well as in the more familiar use of

the nervous system to control muscle activity and thereby control animal movement in 3-dimensional space (Figure 1A). Thus, morphogenetic control and behavior are isomorphic in the sense that in both cases, bioelectric signaling coordinates activity, of cells or muscle groups respectively, in a continuous space of possibilities which must be mapped and represented at some level of detail for adaptive outcomes (Figure 1). This view of nervous systems as functionally continuous with non-neural signaling systems is consistent with the gradual and clade-specific elaboration of nervous-system structure suggested by considerations of late-Cryogenian to early-Ediacaran ecology (e.g. Erwin, 2015).

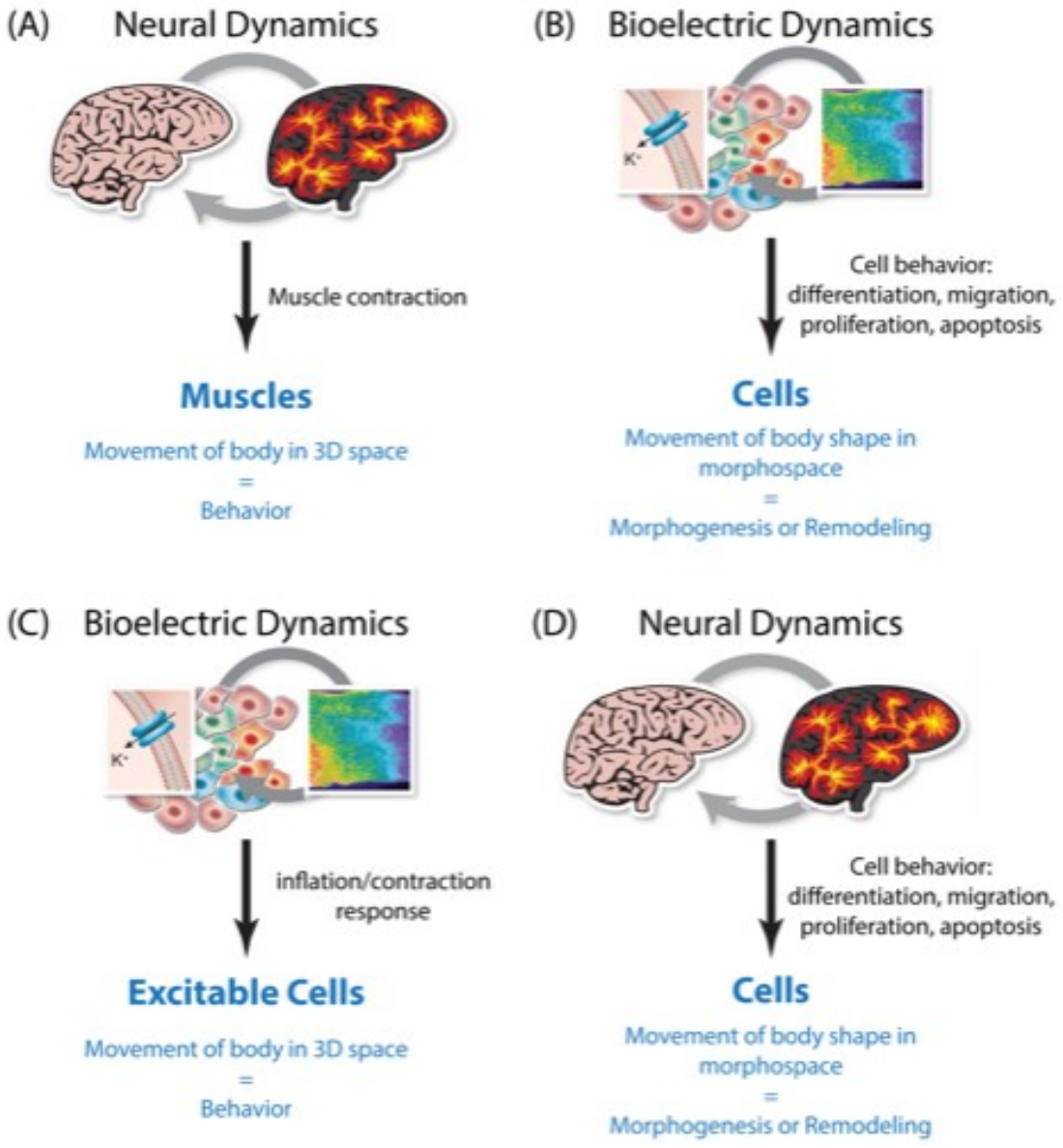

Figure 1: Both neural and bioelectric networks are feedback controllers that guide trajectories through different spaces. (A) In the familiar case of animal behavior across eumetazoa, neural networks represent 3D spaces, issue commands to muscles, and thus control the movement of the animal through that space in order to reach adaptive goals. Neuroanatomy (left image) and neural function (right image) are mutually enabling and stabilizing (red circular arrow). (B) The same scheme is used by

an ancient morphogenetic system in which bioelectric networks issue commands to cells, modifying the structure of the body during embryogenesis or regeneration to guide it through a morphospace of possible configurations to reach a specific anatomical outcome. Cellular network architecture (left image) and the resulting spatial gradient of bioelectric states (right image) are again mutually enabling and stabilizing (red circular arrow). Importantly however, the shared evolutionary history of these systems and the ancient roles of bioelectric circuits enable the converse as well: organisms such as glass sponges use non-neural bioelectrics to coordinate their pulsatile behavior (C), while vertebrates use brain and CNS tissue to help coordinate developmental patterning (D). Pseudo colored image of a tadpole flank in the presence of a small molecule fluorescent dye obtained from Dr. Douglas Blackiston, used with permission. The other panels are courtesy of Jeremy Guay of Peregrine Creative Inc., used with permission.

In what follows, we develop, consider the evidence supporting, and discuss some implications of the hypothesis that morphological coordination is the evolutionarily-primary function of nervous systems. The next section expands the notion of an “option space” for early nervous-system evolution (Jékely, Keijzer and Godfrey-Smith, 2015) to include neural control of cell proliferation and neural control of cell differentiation, functions that enable the exploration of abstract morphological and cell-fate spaces, respectively. It then provides a general statement of the coordination problem and examines its formal structure from a control- and network-theoretic perspective. We next review the neural coordination solutions adopted by metazoa, from the preneural signaling systems of sponges, through the development of specialized nerve nets and proto-ganglia in cnidarians and ctenophores, to the development of a central nervous system (CNS) and

encephalization in bilaterians. We consider experimental evidence for the control of morphogenesis by neural signaling during development, regeneration, and tumorigenesis, and for the integration of such neural signals with small-molecule and non-neural bioelectric signaling in these settings. Finally, we suggest some potential implications of these findings for both research and therapeutic strategy in birth defects, cancer, and regenerative medicine and consider possible applications in bioengineering.

The problem of coordination: from cells to bodies

The multicellular lifestyle long predates eukaryotes, having been developed by the cyanobacteria and purple sulfur bacteria some 3,700 Mya (Stal, 2012; Nutman et al., 2016). The layered morphology of microbial mat communities is driven by resource availability and physical constraints such as gravity (Stal, 2012; Prieto-Barajas, Valencia-Cantero and Santoyo, 2018). The hierarchical branching morphologies of plants (Prusinkiewicz, 1998; Li et al., 2017) can also be understood in terms of resource and physical constraints, as demonstrated by the strikingly plant-like forms obtained by regulating the access of inorganic feedstock materials to CO_2 (Turner and Nottale, 2017). While animal morphologies are clearly responsive to such resource and physical constraints, the presence of both symmetries and specific asymmetries between distant parts of the body indicates the action of additional self-imposed long-range constraints acting between specific target sites. The combination of left-right (L-R) symmetries and anterior-posterior (A-P) asymmetries typical of bilaterian limbs, for example, cannot be explained solely in terms of resource constraints or physical forces such as gravity. Animal morphologies specifically raise the question of how information specifying long-range constraints on cell proliferation, differentiation and behavior is transmitted from one part of a multicellular body to another.

Motivated in part by the skin-brain hypothesis (Keijzer, van Duijn and Lyon, 2013), Jékely, Keijzer and Godfrey-Smith (2015) proposed that nervous-system evolution be viewed as solving two kinds of problems simultaneously: input-output problems typified by sensory-motor coordination, and internal coordination problems typified by the coordination of muscle-based motility. Problems of the former type generally involve moving information from one tissue to another, while those of the latter type involve moving information within a tissue. While Jékely, Keijzer and Godfrey-Smith (2015) consider roles for neural signaling in controlling physiological and developmental processes, they focus on the whole-organism scale in defining their "option space" for nervous-system evolution. We suggest that these same options should also be considered at the scale of single cells or small populations of cells, i.e. that nervous system evolution can also be viewed as addressing the problems of coordinating the proliferation, differentiation, and motility decisions of these smaller units. In principle, neural signaling could be involved in transferring information that coordinates or controls such processes between cells of the same type or within the same tissue; as we will see, however, it is the capability to effect precise coordination between cells of different types over longer than typical nearest-neighbor distances that nervous systems add to the signaling capabilities already developed by facultatively multicellular systems.

In order to state the morphological coordination problem faced by multicellular animals in a general form, it is useful to adopt the concept of a "target morphology" for a species, the stable endpoint that typical development (or in competent species, regeneration) achieves in that species, averaged over individual differences (Levin, 2011). Armed with this concept, we can state the *morphological coordination problem* as the problem of achieving those aspects of the target morphology that cannot be achieved by a combination of cell-autonomous processes, local responses to

resource and physical constraints and common, local rules executed everywhere.

As an example of the morphological coordination problem, consider the problem of assuring overall L-R symmetry in a vertebrate body while breaking that symmetry in the precise ways needed to construct the appropriate anatomy. Growth rates of vertebrate cells depend on local concentrations of growth factors and other morphogens as well as on local energetic resources; none the less, the sizes and shapes of even distal appendages such as human fingers are roughly L-R symmetric. While equivalent, purely-local regulation on left and right sides may explain structural symmetries, e.g. equal numbers of fingers, small differences in initial conditions, noise, and competition for resources between cell populations on opposite sides could all introduce asymmetries in cell proliferation rates and hence the sizes of left and right structures. Specific L-R asymmetries, e.g. of the heart, liver, spleen, gut, and brain, are universal (Neville, 1976; Palmer, 2009), and L-R symmetry is broken as early as the first embryonic cleavage to assure L-R asymmetric development (Levin, 2006; Vandenberg, Lemire and Levin, 2013). How, then, are the equal sizes and shapes of structures that are L-R symmetric, such as limbs, assured during embryonic and later development? How is cell proliferation coordinated across the L-R axis? Experiments assessing the response of *Xenopus* embryos to induced tumorigenesis indicate that L-R symmetry of overall cell proliferation rates is enforced by long-range bioelectric signaling as early as the 16-cell stage, before the nervous system begins to develop (Chernet, Fields and Levin, 2015). The proliferation, and hence the states, of cells on one side of the embryo depend, in this case, on the states of cells on the other side: precisely the kind of long-range conditional dependence that cannot be achieved with purely local models of development.

In order to formalize this distinction between developmental processes supported by only short-range versus both short- and long-range signaling, we abstract cells to communicating agents and represent the network of cell-cell signaling as an abstract constraint-communication network (Friston, 2013). The distinction between constraint networks supporting only local or both local and non-local conditional dependencies can be quantified by the average path length between nodes. In a spherically-symmetric network with only nearest-neighbor connections, the average path length is one-half of the graph-theoretic diameter, i.e. one-half of the maximum minimal path length in the network. In a complete (i.e. every node is connected to every other node) network, the average path length is one. Between these extremes are networks in which a relatively small number of long-range connections connect clusters of locally-connected nodes; these are small-world networks (SWNs; Watts and Strogatz, 1998; Barabási and Albert, 1999), defined as networks with both a small average distance between nodes and a high clustering coefficient as shown in Fig. 2. Such networks are efficient, in the sense of providing short average path lengths and thus allowing fast long-distance communication with a relatively small (compared to a complete graph) total number of connections (Latora and Marchiori, 2001). Like random graphs, they allow most nodes to be identified by their connection patterns (in the limit of a sufficiently large graph, every node has a unique connection pattern), a form of identification that is lost as the graph approaches a complete graph.

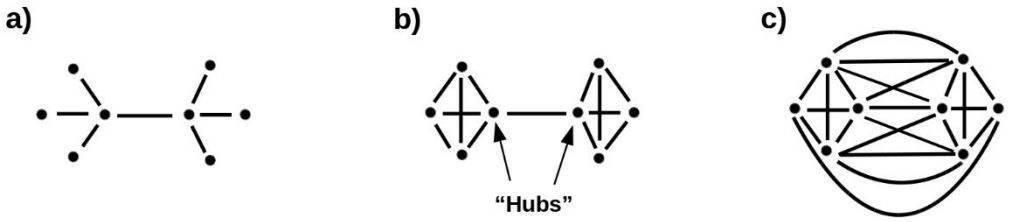

Figure 2: a) A network with no closed paths and hence a clustering coefficient of zero; this network is not an SWN. b) A simple SWN with two distinct local neighborhoods of mutually connected nodes connected by two "hub" nodes. Note that each local neighborhood contains closed paths that decrease the average distance between nodes. The closed paths give it a non-zero clustering coefficient. c) Adding more paths to the SWN in b) destroys the "small world" structure. This network is almost complete, i.e. every node is connected to almost every other node. In a complete network, the nodes are not distinguishable by their connectivity. Connections between nodes can in all cases be interpreted as non-directional, directional, or bidirectional, incorporating feedback. Adapted from Fields and Glazebrook (2017).

Small-world networks are ubiquitous in biological systems, e.g. in gene regulation (Agrawal, 2002), protein interactions (Barabási & Oltvai, 2004), human neurocognitive architecture (Bassett & Bullmore, 2006), academic collaborations (Newman, 2001) and other social-exchange networks. In many cases, e.g. human neurocognitive architecture, constraint networks are structured hierarchically as SWNs of SWNs (e.g. Sporns and Honey, 2006). Such networks are capable of communicating both positive and negative constraints, e.g. both positive and negative regulatory signals in protein-

interaction networks or both excitatory and inhibitory signals in neural networks; such parallel constraint communication enables both positive and negative feedback signals. In all cases, such constraints act locally; however, their sources are the states of distant nodes in the network. If organisms employ long-distance communication between cells or tissues to solve the morphological coordination problem, one would expect, on grounds of thermodynamic efficiency, the architecture supporting this communication to be a SWN. One would hence expect the architectures of even rudimentary nervous systems to be or at least include SWNs. Evidence that the nervous systems of both ctenophores (Moroz, 2015) and cnidarians (Galliot and Quiquand, 2011; Kelava, Rentzsch and Technau, 2015) exhibit neuron clustering and may comprise multiple, interconnected subnetworks suggest that this expectation is correct. As a driver for the evolution of nervous systems, the communication efficiency of SWNs (Latora and Marchiori, 2001) would support their independent origin in multiple lineages and hence convergent functional evolution, as has been suggested in the case of ctenophores on the basis of primarily biochemical evidence (Moroz, 2015; Moroz and Kohn, 2015).

Nervous system structure correlates with morphological complexity in Eumetazoa

What is a nervous system?

Nervous systems comprise neurons. The definition of a neuron, however, remains controversial, with typical structural definitions, e.g. in terms of synapses, requiring exceptions (e.g. Nickel, 2010; Bucher and Anderson, 2015; Jékely, Keijzer and Godfrey-Smith, 2015). Most, if not all, cells are able to change their electrical properties, using the same ion channels and downstream neurotransmitters and calcium as second messengers that neurons use – these are ancient functions that pre-date specialization of

neurons for speed and selective connectivity. Motivated by the network-theoretic considerations above, here we define neurons functionally in terms of specificity and speed: a neuron is a cell that transmits electrical or chemical signals from one or more specific source cells to one or more specific target cells in a time much less than that required for source-to-target diffusion. A nervous system is a network of such cells connected in a way that allows longer-distance signaling and signal processing. Note that this definition applies even if the source-target connections are randomly chosen and short as in the model of de Wiljes et al. (2017), provided the signals are transmitted specifically from the randomly-chosen source to the randomly-chosen target with a transmission speed that exceeds the diffusion speed across the same distance.

Genetic toolkits for instructive signaling and body-axis specification predate complex morphology and neurons

Phylogenomic methods allow the correlation between nervous systems and morphological complexity found in the Eumetazoa to be probed at the molecular scale to investigate whether newly developed nervous systems and complex morphology were enabled by the introduction of novel signaling or regulatory systems in the Eumetazoa. Consensus data show that a broad range of eumetazoan hormones, including insulin, adrenocorticotropine (ACTH), triiodothyronine (T3), and prostaglandin (PGF2), and growth factors predate the divergence between unikonts, including the Metazoa and Amoebozoa, from bikonts, including ciliates and plants (Lenard, 1992; Csaba, 2012). The eumetazoan neurotransmitters acetylcholine, dopamine, norepinepherin, and serotonin as well as many eumetazoan synapse-associated channels, ion pumps, GTPases and other signaling-system components are similarly broadly distributed across the phylogenetic tree (Csaba, 2012; Roshchina, 2016; Burkhardt and Sprecher, 2017; Plattner and Verkhratsky, 2018). Prindle et al. (2015) show, for example, that long-range

K^+ ion channel-mediated communication is employed for global metabolic coordination in *B. subtilis* biofilms. Both the availability and use of these molecules for intercellular signaling to control cell proliferation and cellular behavior thus predates even the advent of obligate multicellularity, not to mention the development of nervous systems or complex morphology.

In the same way, signaling systems employed by neurons were available even to unicellular eukaryotes. Signaling factors directly involved in the specification of characteristic features of animal morphology, e.g. multiple distinct body axes with specific symmetries and asymmetries, predate nervous systems. One well-explored such factor is the Wnt pathway, which is centrally involved in primary body axis specification across all metazoa but is represented only in precursor form outside the metazoa (Holstein, 2012; Adamska, 2015; Tweet and Irwin, 2015; Loh, van Amerongen and Nuss, 2016). The facultatively multicellular *Dictyostelium* offers an interesting comparison here, as it expresses Wnt pathway homologues only during the multicellular stage (Harwood 2009). One can speculate about which of the dual roles of the downstream Wnt effector β-catenin – cell adhesion or signaling – arose first and which was co-opted later, but it is likely not a coincidence that these same molecules are involved in both establishing tissue planar cell polarity (long-range order providing information on *direction*; e.g. Wallingford and Mitchell, 2011) and axial patterning (information on *position* along the organism; e.g. Pietak et al., 2019). Larger numbers of distinct Wnt subfamilies and associated regulators exist in eumetazoa than in placazoans or sponges, suggesting that Wnt pathway expansion may be associated with more complex metazoan morphologies (Loh, van Amerongen and Nuss, 2016); however, the question of subfamily loss in basal metazoa remains open. In Cnidarians and Bilaterians, the secondary dorsal-ventral (D-V) axis is specified by TGFβ/BMP pathway signaling; however, this pathway also exists in both sponges and placozoa and presumably plays a role in oral-arboral axis specification in these

organisms (Adamska, 2015; Tweet and Irwin, 2015; see DuBuc, Ryan and Martindale, 2019 for recent data from placozoans). Other signaling systems once thought to be specific to eumetazoa, including the Hox system, have also been found in the morphologically-simpler basal metazoa (Adamska, 2015; Tweet and Irwin, 2015).

Moreover, neither neurons nor multicellular morphology are required to support whole-body sensation and behavior. This is clearly the case in unicellular predators such as *Paramecium*, which move using cilia beat driven gliding and reverse their movement upon hitting an obstacle using membrane depolarization and resulting reversal of the cilia beat pattern, allowing them to exhibit complex behaviors (Machemer and Eckert, 1973). Similar examples of complex behaviors in the absence of a specialized nervous system are apparent as well in bacteria, plants, and many types of individual somatic cells and multicellular tissues (Baluška and Levin, 2016). Facultative multicellular systems such as *Dictyostelium*, as well as the relatively soft-bodied demosponges, employ paracrine signaling (Nickel, 2010) with a bioelectric response (Leys, 2015) to coordinate whole-body contractile movements in response to stimulation. The syncytial glass sponges (Hexactinellida) employ electrical signaling, including whole-body action potentials, to accomplish the same thing (Leys, 2015).

Overall, this evidence suggests that most signaling factors, be they hormones, morphogens or neurotransmitters, are widespread across phyla and predate obligate multicellular life, indicating that neurons are not intrinsically required in simple animals for the functions they currently fulfill. Specialized cell types are predated by complex signaling functions, behaviors and the required toolkits. Why then do eumetazoa have neurons? As suggested above, geometry, not biochemistry, may be the answer. We hypothesize that with the advent of multicellular bodies, even if these have a primarily behavioral or protective function (Fields and Levin, 2019), the

nervous system developed to transmit some of the previously developed signaling factors over longer than typical nearest-neighbor distances to allow for large scale coordination of cell proliferation, sensory processing, behavior, morphological development and differentiation in complex animals (Figure 3).

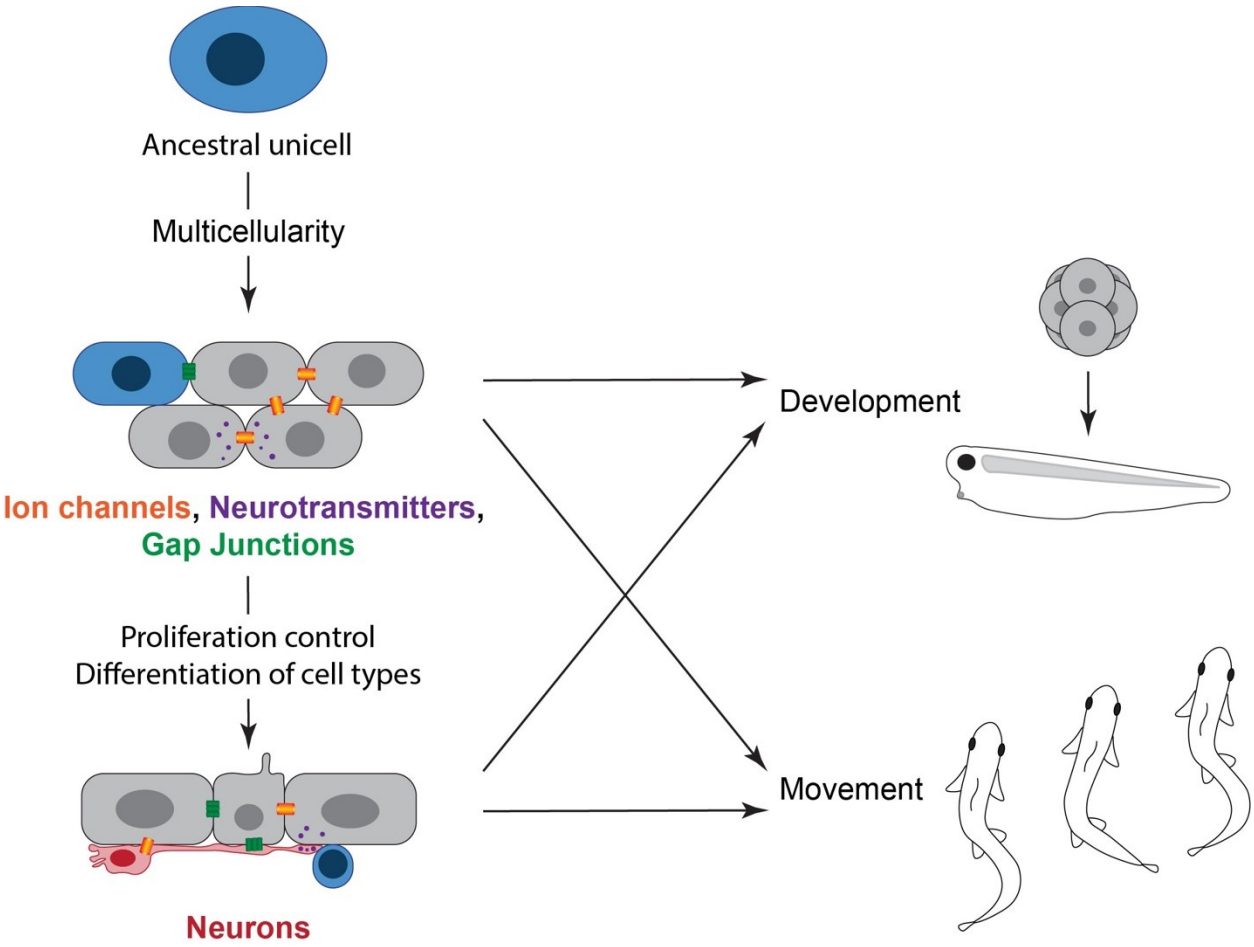

Figure 3: Proposed model in which bioelectric machinery (ion channels, neurotransmitters and gap junctions) and cell type differentiation developed successively and have both been adapted to control developmental patterning and movement. Alongside this process, proliferation control was developed, restricting stem cell properties (blue cell).

Nervous system evolution: Once, twice, or often?

While there is broad support for Cnidarians and Bilaterians being sister clades, the relationship between the Porifera, Placozoa, and Ctenophores remains controversial, with some molecular analyses supporting Porifera (Feuda et al., 2017; Simion et al, 2017;) and others supporting Ctenophores (Liebeskind et al., 2017; Whelen et al., 2017) as the earliest metazoan branch (see Botting and Muir, 2018 for an argument from the fossil record that sponges did not appear until the Cambrian). The placement of placozoans is similarly uncertain. Ultrastructural and physiological evidence suggesting that the choanocytes of demosponges (or choanoblasts of glass sponges; Leys, 1999) are closely related to choanoflagellates, the well-established sister group of the Metazoa (Funayama, 2013; Sebé-Pedrós, Degnan, and Ruiz-Rillo, 2017), provide additional support for early branching of Porifera; however, the question of early metazoan phylogeny remains unsettled.

If the aneural Porifera and Placozoa are the most basal metazoan branches, nervous systems can be considered a synaptomorphy of the Eumetazoa (Galliot and Quiquand, 2011); any other evolutionary scenario, however, requires either multiple origins of nervous systems or loss of nervous systems in Porifera and Placozoa (Moroz and Kohn, 2015). The nervous systems of ctenophores differ sufficiently from those of cnidarians and bilaterians, moreover, to suggest their independent origin under any phylogenetic placement (Moroz, 2015; but see Arendt, Tosches and Marlow, 2016 for a dissenting view). The extreme diversity of neural architectures in bilaterians raise similar questions (Northcutt, 2012). Nervous systems in the early-branching Xenacoelomorpha (Cannon et al., 2016), for example, range from simple nerve nets with no apparent ring or ganglion centralization to systems with recognizable anteriorly-located brains (Gavilán, Perea-Atienza and Martínez, 2016; Martínez et al., 2017). Whether this variation represents, when compared with other bilaterians, loss or convergent development of an anterior brain remains unknown; a similar question

concerning centralization arises when comparing the more rudimentary examples of Xenacoelomorph nervous systems to the more organized examples from cnidarians.

Is the neuron itself the principal innovation enabling complex morphology?

Neurons offer a key advantage over either paracrine or electrical signaling by non-neural cells: neurons are able to deliver a signal from a single source to a specific distant target. Unlike organism- or tissue-scale morphogen gradients or electric fields, or even cell-type specific hormonal signals, neurons enable long-range signaling at single-cell or even subcellular resolution. From an evolutionary point of view, neurons are the best biological solution to date for spatially- and temporally-precise information transfer and processing.

How did this innovation in signaling precision come about? Nickel (2010) suggested that interstitial cells in demosponges, which are capable of receiving and then relaying paracrine signals, can be regarded as precursors of neurons; concentrating signaling molecules in extended processes of such cells would improve the spatial resolution of signaling. Sponges lack both muscle and neurons, and hence do not have “skin brains” as originally defined (Keijzer, van Duijn and Lyon, 2013); however, sponge epithelia are capable of coordinated, bioelectrically driven contractions (Leys, 2015) and can be viewed as partial precursors of neuron-coordinated myoepithelia (Keijzer and Anellos, 2017). Funayama (2013) advances a model in which the archeocyte stem cells of demosponges, which have amoeboid morphology and are mobile within the interstitial space, are descendants of a mobile, amoeboid alternate form of ancestral choanocytes, a suggestion consistent with environment-dependent transitions between flagellated and amoeboid forms in unicellular eukaryotes (Brunet and King, 2017) as well as recent molecular evidence (Sogabe et al., 2019). A signal-relaying function has not

been directly established for archeocytes; however, it seems plausible that stem cells, which must both send and receive signals that induce proliferation and differentiation, might also serve as general signal-relaying cells in adult multicellular systems without neurons. Such stem-cell-derived, or relayed signals may serve the same functions in constructing and maintaining target morphology in adults that they serve in embryogenesis. Mesenchymal stem cells, for example, are known to secrete cytokines that act broadly on immune-system cells (Kyurkchiev et al., 2014) as well as angiogenic and growth factors capable of modulating tissue regeneration and vascularization (Tran and Damaser, 2015).

If neurons are involved in morphological coordination as suggested here, one would expect neurons to be one of the earliest-differentiating cell types in embryogenesis. Although embryos undergo complex morphological rearrangements prior to the appearance of differentiated cells, in most bilaterians neurons differentiate from epithelial cells of the early embryo at the time of gastrulation (Hartenstein and Stollewerk, 2015). In planaria, which do not undergo gastrulation, the nerve cord is the initial adult structure produced in the embryo (Martín-Durán, Monjo and Romero, 2012). Neurons are similarly among the first evident differentiated cells in acoels (Ramachandra et al., 2002) and cnidarians (Galliot and Quiquand, 2011; Piraino et al., 2011) in which embryonic development has been characterized.

Nervous systems enable error correction

Morphological invariance across generations requires a heritable encoding of target morphology. Where is this information stored, and how is it inherited? Both theory and experiment indicate that it cannot be wholly stored in the genome, even with epigenetic modifications; the organization of the cytoplasm, cytoskeleton, and membrane of the ovum and zygote also carry

essential information about target morphology (Fields and Levin, 2018). This information must, moreover, be encoded with sufficient redundancy to enable error detection and correction. In *Micrasterias*, for example, the coupling between membrane receptors and cytoskeleton enables information stored structurally and bioelectrically to be mutually error-correcting (Fields and Levin, 2018). Similarly, a classical example of epigenetics demonstrated stable (permanent) propagation of changes in cellular anatomy in lines of ciliates by mechanical perturbation of the membrane cytoskeleton without genetic change (Beisson and Sonneborn, 1965; Nelsen, Frankel and Jenkins, 1989).

Neurons, we suggest, similarly enable error correction in multicellular organisms. The path taken by a signal transmitted by a neuron can be replicated exactly, enabling both redundant communication and back-and-forth handshaking between source and target. The sensory-motor loops enabled by path replication are essential for behavioral coordination (Kandel, Schwartz and Jessell, 1981); we hypothesize that they play a role in morphological coordination as well.

Long-range morphological coordination requirements predict nervous system complexity

The transition from diffuse “nerve net” architectures through local modules, e.g. nerve rings and nerve cords, with few long-range connections to hierarchies of small world networks (SWNs) as morphological and behavior complexity increases has been noted previously (Kaiser and Varier, 2011; Bauer and Kaiser, 2017). The execution of complex behaviors clearly requires the coordination of mutually-distant parts of the body, e.g. of multiple limbs for locomotion. We suggest here that information transfer between different parts of the developing body is essential to the production of the invariant adult morphologies typical of Eumetazoa, and that nervous

systems are the evolutionary innovation that enables such information transfer at single-cell resolution. We further suggest that the requirements for long-distance, high-resolution information transfer increase with the number of specifically symmetric (e.g. left and right index fingers in humans) or asymmetric (e.g. right thumb versus right big toe) structures. Animal phylogeny can be viewed, in this case, as an elaboration of bodies enabled by an elaboration of nervous systems. The increase in nervous system complexity from the nerve nets of Cnidarians to the ring and nerve cord structures in roundworms or the brain and nerve cord organizations in flatworms and further to insect, cephalopod and chordate nervous systems is well documented (Holland et al., 2014; Arendt, Tosches and Marlow, 2016), as is the elaboration of mid- and forebrains in vertebrates (Northcutt, 2002). Eumetazoa with reduced brains compared to their phylogenetic peers are considered instances of niche-dependent brain loss (Hirth, 2010), with reduction to only a nerve net consistent with a rudimentary bilateral body plans (Gavilán, Perea-Atienza and Martínez, 2016).

In both cephalopods (Albertin et al. 2015) and chordates (Pendleton et al., 1993; Tepass et al., 2000; Holland, 2009) the increase in both morphological and nervous system complexity is accompanied by expansion of gene families responsible for cell-cell recognition as well as transcription factors. Differential expression studies in multiple model systems have consistently shown that many if not most members of such regulatory gene families are expressed in the nervous system even when they are also expressed elsewhere (Adams, Kerlavage, Fields and Venter, 1993; Adams et al., 1995; Brown et al., 2014; Kang et al., 2011; Moroz et al., 2006).

High-resolution neural signaling complements lower-resolution molecular and bioelectric signaling in development and regeneration

The importance of coordinated neural activity in the development of the brain and other central nervous system (CNS) structures has been well appreciated since the pioneering work of Wiesel and Hubel (1963a;b) and is encapsulated in Hebb's dictum that “neurons that fire together wire together” (Kandel, Schwartz and Jessell, 1981). Outside of the CNS, however, the role of specific neural signaling in controlling differentiation and morphogenesis has received far less attention than the roles of molecular and non-neural bioelectric signaling. Classic studies from the 19th to mid 20th centuries showed that denervation disrupted regeneration in amphibians, but only more recently have these effects been extended to other systems or studied at cellular and molecular resolution (reviewed by King and Newmark, 2012; Kumar and Brockes, 2012; Farkas and Monaghan, 2017). Here we review studies indicating a role for neural activity in normal development, including development by regeneration in asexually-reproducing systems such as *Hydra* or planaria.

Neural activity in invertebrate development and regeneration

The Cnidarian *Hydra* has a nerve net with ring-like specializations but no well-defined brain (Koizumi, 2002; Galliot and Quiquand, 2011). Hydra reproduce sexually but are also capable of asexual reproduction via budding, regeneration from small fragments, and reassembly following disaggregation (Watanabe, Hoang, Mättner and Holstein, 2009). Differentiation of both head (oral) specific neurons and non-neural head-specific epithelial structures is induced by morphogenic peptides secreted by anterior neurons (Schaller, Hermans-Borgmeyer and Hoffmeister, 1996). Epithelial cells can, however, also assume this inducing role in *Hydra* from which neurons have been selectively removed, by expressing normally neuron-specific genes (Wenger, Buzgariu and Galliot, 2015). Hence while the signaling functions of neurons can be adopted by other cells in Hydra, these typically-neural functions,

however they are implemented, contribute to the specification of even non-neural morphology.

In contrast to Hydra, planaria have well-developed brains and chemical synapses (Pagán, 2014; Cebrià, 2008); while obligate-sexual species exist, many others reproduce asexually via fission and regeneration (Elliott and Sánchez Alvarado, 2012). In the planarian *D. japonica*, fragment-scale bioelectric (membrane voltage, V_{mem}) asymmetry is the first known instructive signal for anterior-posterior axis definition and is sufficient for normal regeneration in fragments containing roughly 20% of the original ventral nerve cord (Durant et al., 2019); whether neural activity amplifies small V_{mem} differences to efficacious levels and whether this V_{mem} signal is sufficient in much smaller but still regeneration-competent fragments is not yet known. In small fragments containing only lateral branches originating from the VNC, however, the orientation of neural processes determines the orientation of the new A-P axis, indicating that neural polarity is critical for axis definition and subsequent morphogenesis (Figure 4). Blockage of microtubule-based motor transport disrupts axis specification, suggesting that neurons can regulate morphogenesis not just via chemical or electrical synaptic transmission but also via active, directional transport of morphogens (Pietak et al., 2019).

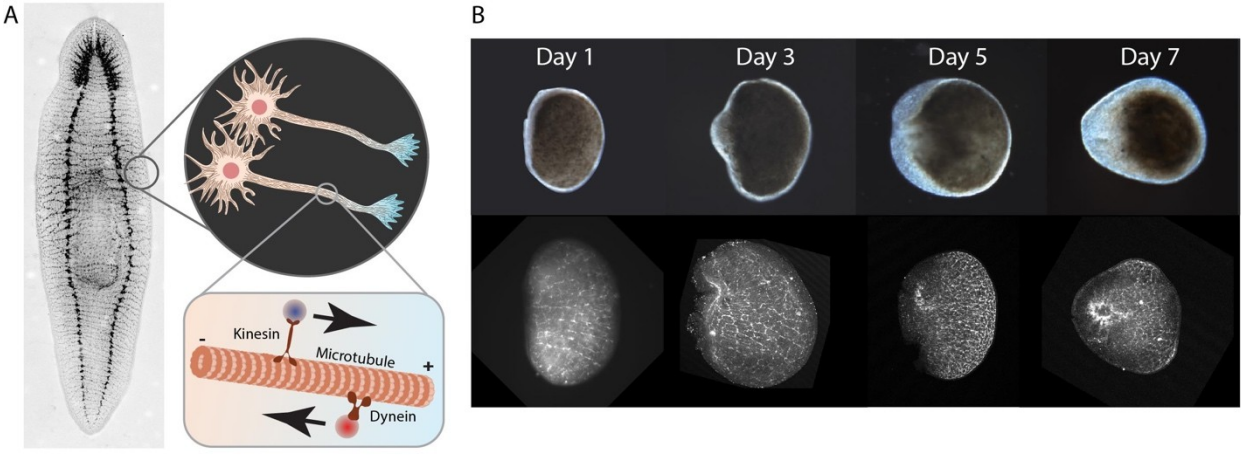

Figure 4: Neural transport of morphogens sets anterior-posterior axis in regenerating planaria. A) Planarian nervous system as visualized with synapsin antibody labelling. The model detailed in Pietak et al., 2019 proposes transport of morphogens via molecular motors along the microtubules of the axons. B) Lateral fragments of a planaria, which contain only neurons oriented perpendicular to the original anterior-posterior axis, regenerate with a new anterior-posterior axis aligned with the orientation of the nerves in the fragment, perpendicular to the original axis; this confirms the prediction of the model, that neural directionality sets the anatomical pattern (over-riding and re-setting prior anterior-posterior chemical gradients). Schematic in panel A and images in panel B are used with permission from Pietak et al., 2019.

In the roundworm *C. elegans*, generally considered a paradigm case of cell-autonomous development, the two canal-associated neurons (CANs) have been shown to regulate the number and placement of vulvae by regulating Wnt signaling (Modzelewska et al., 2013). While these neurons have gap junctions (Altun, Chen, Wang and Hall, 2009) and hence presumably employ electrical signaling, their role in morphogenesis appears to be mediated by spatially-localized morphogen secretion. In *Drosophila,* local larval neurons that persist through metamorphosis guide axons of adult neurons from segment-specific imaginal discs into the developing CNS (Usui-Ishihara, Simpson and Usui, 2000; Williams and Shepherd, 2002). Again, this effect appears to be via secreted morphogens that act neurotropically.

Neural activity instructs morphogenesis in Xenopus embryos

Studies of direct neural effects on normal development have focused on the frog *Xenopus*. Micro-injury to the spinal cord in the frog embryo results in characteristic defects in the tail (Modia et al., 2011). Moreover, removal of the nascent brain in Xenopus induces a range of posterior tissue phenotypes,

including disorganization of the trunk musculature and peripheral innervation, revealing that inputs from the brain are required for normal morphogenesis, even in regions distant from the brain (Herrera-Rincon and Levin, 2018; Herrera-Rincon et al., 2017) (Figure 5). Thus, some somatic structures are not purely locally-determined but require instructive influence from distant locations. The developmental defects induced by brain removal can be partially ameliorated by induction of bioelectric and neurotransmitter signaling in other non-neural tissues; however the rescue is incomplete. This shows that the mechanisms mediating these influences can also function in non-neural cells (representing a pre-neural function; Levin, Buznikov and Lauder, 2006) but that full efficiency towards the correct anatomical specification is best accomplished by the optimized CNS.

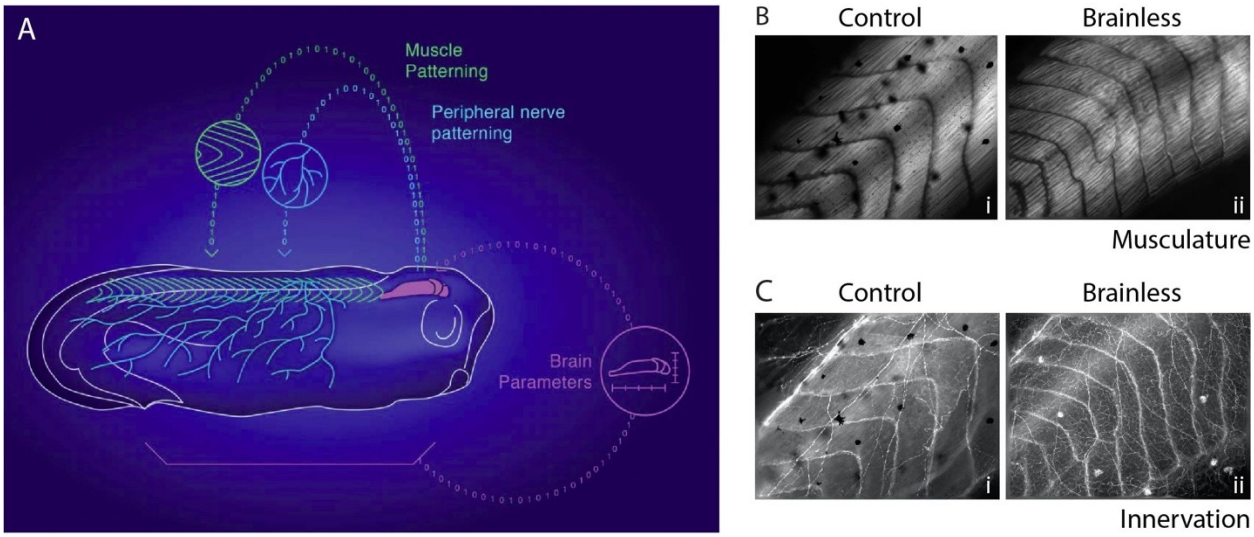

Figure 5: The brain is required for normal embryonic development. A) Overview of the systems effected by brain removal. B) Removal of the brain interferes with the correct patterning of musculature when compared between control animals (i) and brainless animals (ii). C) Brain removal also impacts peripheral nerve patterning in brainless animals (ii) compared to controls (i). Panels A and B,C are used with permission from Herrera-Rincon and Levin, 2018 and Herrera-Rincon et al., 2017 respectively.

A role for neural signaling in developmental disorders?

The results on CNS-mediated effects on morphologically-correct development found in *Xenopus* embryos (Herrera-Rincon and Levin, 2018; Herrera-Rincon et al., 2017) clearly must be more fully characterized and extended to other systems before they can be generalized. Tentative as they are, however, they immediately raise the question of whether electromagnetic or biochemical disruption of CNS function in late embryogenesis and/or early fetal development in humans could have not just proximal effects on CNS development but also distal effects on morphology. The association of morphological symptoms with neurodevelopmental disorders such as autism (Ozgen et al., 2011) and teratogens such as ethanol, many of which act first or primarily on the developing CNS (de la Monte and Kril, 2014), as well as the numerous channelopathies (ion channel mutations) that exhibit both, neurological and anatomical phenotypes (e.g. Dahal et al., 2012; Masotti et al., 2015; Tristani-Firouzi and Etheridge, 2010) suggests that evidence for such distal effects of early neural activity disruption may be already available, but so far remain uninvestigated.

The apparently normal gross morphology of the lower body in surviving anencephalic neonates with only a rudimentary brainstem (Poretti et al., 2010) suggests that cerebral structures are not required for overt, posterior morphogenesis in humans; however, the very short lifespans of such neonates, their lack of organized behavior, and the lack of histological data on the morphogenesis of other organs in these cases preclude detection of dysmorphias. The role of the central nervous system in the maintenance of normal morphology in humans or other mammals through the lifespan remains uninvestigated.

Innervation in tumor growth and metastasis

The role of growth factors and other small-molecule morphogens in cancer is well-established, and the role of non-neural bioelectric signaling is increasingly understood (reviewed by Chernet and Levin, 2013). Early experiments in model systems indicated that denervation could induce tumorigenesis, suggesting that normal neural activity plays a role in suppressing transformation and/or dysregulated proliferation (Chernet and Levin, 2013). Illustrating the diversity of cancer types, other studies have demonstrated an active role for innervation in tumor induction, maintenance, and metastasis, suggesting that tumorigenesis is at least in some cases nerve-dependent in a way analogous to regeneration (reviewed by Boilly et al., 2017; Kuol et al., 2018). A variety of tumor cells are stimulated by neurotransmitters, although dopamine can have a tumor suppressive effect (Moreno-Smith et al., 2011). Tumor cells also secrete tropic factors that induce innervation from surrounding normal tissue (Boilly et al., 2017), analogous to the induction of vascularization. Hence different tumor cells in different environments respond in different ways to neural stimulation.

These results suggest that if neural activity contributes to the regulation of cell proliferation, as developmental data suggest, this regulation can be over-ridden by tumor cells. The common dependence of regeneration and tumorigenesis on inducible stem-cell proliferation, the inverse relationship between regenerative capability and cancer susceptibility across phylogeny (Smetana, Dvořánková and Lacina, 2013), and the apparently common mechanisms of nerve dependence in regeneration and tumorigenesis (Boilly et al., 2017) all suggest a continuous, bidirectional flow of information between stem cells and neurons in the healthy case. If stem cells and neurons have common epithelial origins as suggested (Funayama, 2013; Arendt et al., 2015; Arendt, Tosches and Marlow, 2016), this communication pathway may serve to regulate morphogenesis and morphological maintenance across the metazoa.

Discussion

Here we have presented theoretical arguments and reviewed data supporting an active role for nervous systems in coordinating animal morphogenesis. We highlighted the evolutionary origin of nervous system functions in ancient biochemical and bioelectric mechanisms that were co-opted and scaled up from the control of cell behaviors in the environment of the body to the control of animal behavior in its macro environment. We have suggested, in particular, that the Precambrian development of nervous systems enabled the development of complex, multi-axis animal bodies and hence the Cambrian explosion. Such bodies in turn enable the complex, neurally-controlled behaviors typical of animals, including predation, escape, social communication, and active mating behaviors.

While the development of the nervous system *per se* has been studied for decades in many model systems and the neuron-dependence of regeneration is well known, experimental investigation of the role of neural activity in morphogenesis has barely started. Emerging evidence that innervation modulates tumor growth and that disruption of normal neural activity may have morphological as well as neurological consequences lends urgency to this area of research. We expect that neural signaling will become recognized as complementing morphogen and non-neural bioelectric signaling in the control of both morphology and differentiation, just as it does in the control of motility and behavior. Likewise, non-neural bioelectric and neurotransmitter signaling is increasingly recognized as an instructive long-range coordination system for guiding cell behavior during remodeling, regeneration, embryogenesis, and cancer suppression (Levin and Martyniuk, 2018; McLaughlin and Levin, 2018).

We propose a conceptual parallel between the roles of the brain and of non-neural bioelectric networks. Evolution discovered, as early as in bacterial

biofilms (Liu et al., 2017; Prindle et al., 2015), the benefits of exploiting the physics of electrical processes for computation and coordination (Pietak and Levin, 2017). The functions of non-neural bioelectric networks in guiding cell behaviors toward a specific attractor state in the animal's morphospace were speed-optimized and used to enable animal bodies to achieve behavioral goals in the development of the nervous system (Levin, 2012; Pezzulo and Levin, 2015). This view has significant implications beyond evolutionary biology, because neuroscience has developed mature models of how physiological networks can optimize system dynamics, ensuring robust functions, despite unpredictable stressors. An appreciation of the evolutionary origin of nervous systems suggests that the tools of neuroscience – both modeling techniques that represent complex biological systems in terms of quasi-hierarchical information flows and experimental techniques that allow direct electrical communication with excitable cells – can be used to target physiological networks underlying the robust, adaptive function of numerous organ systems in health and disease (Pezzulo and Levin, 2015, 2016). Manipulating bioelectrically-mediated decision-making may enable control of complex system-level outcomes (e.g., regeneration of whole organs, immune system regulation, etc.) without the need to directly micromanage transcriptional and translational events. This approach suggests numerous avenues for future investigation at the intersection of the fields of developmental biology and basal cognition. For example, an approach focused on altering cell decision-making, and repairing defects in the sending or receiving of long-range synchronization cues, could exploit both neural and non-neural signals to address cancer reprogramming, birth defects, and traumatic injury (Levin, 2011). More broadly, a recognition of advanced cognitive capacities as evolving from somatic decision-making and pattern memory mechanisms suggests that concepts from information theory and cognitive and behavioral science (Manika and Levin, 2019) could be exploited as novel strategies for regenerative medicine (Matthews and Levin, 2018; Moore, Walker and Levin, 2017), as tools to understand the

development and behavior of simpler animals, and in the construction of new synthetic living machines with desired form and function (Kamm et al., 2018).

Acknowledgements

We gratefully acknowledge support by an Allen Discovery Center award from the Paul G. Allen Frontiers Group (No. 12171), and the Templeton World Charity Foundation (No. TWCF0089/AB55).

References

Adams, D. S. and Levin, M. (2013). Endogenous voltage gradients as mediators of cell-cell
communication: Strategies for investigating bioelectrical signals during pattern formation. *Cell Tissue Res*. 352, 95-122. https://doi.org/10.1007/s00441-012-1329-4

Adams, M. D., Kerlavage, A. R., Fields, C. and Venter, J. C. (1993). 3,400 new expressed sequence tags identify diversity of transcripts in human brain. *Nature Genet*. 4, 256-267. https://doi.org/10.1038/ng0793-256

Adams, M. D. et al. (1995). Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence. Nature 377 (Suppl.): 3-174. PMID: 7566098

Adamska, M. (2015). Developmental signalling and emergence of animal multicellularity. In:
Ruiz-Trillo, I. and Nedelcu, A. M. (Eds.) *Evolutionary Transitions to Multicellular Life*. Dordrecht:
Springer (pp. 425-450). https://doi.org/10.1007/978-94-017-9642-2_20

Agrawal, H. (2002). Extreme self-organization in networks constructed from gene expression data. *Phys. Rev. Lett.* 89: 268702. https://doi.org/10.1103/PhysRevLett.89.268702

Albertin, C. B., Simakov, O., Mitros, T., Wang, Z. Y., Pungor, J. R., Edsinger-Gonzales, E., Brenner, S., Ragsdale, C. W. and Rokhsar, D. S. (2015). The octopus genome and the evolution of cephalopod
neural and morphological novelties. *Nature* 524, 220-224. https://doi.org/10.1038/nature14668

Altun, Z. F., Chen, B., Wang, Z.-W. and Hall, D. H. (2009). High resolution map of *Caenorhabditis elegans* gap junction proteins. *Dev. Dyn*. 238, 1936-1950. https://doi.org/10.1002/dvdy.22025

Arendt, D., Benito-Gutierrez, E., Brunet, T. and Marlow, H. (2015). Gastric pouches and the mucociliary sole: Setting the stage for nervous system evolution. *Phil. Trans. R. Soc. B* 370, 20150286.
http://dx.doi.org/10.1098/rstb.2015.0286

Arendt, D., Tosches, M. A. and Marlow, H. (2016). From nerve net to nerve ring, nerve cord and brain – evolution of the nervous system. *Nat. Rev. Neurosci.* 17, 61-72. https://doi.org/10.1038/nrn.2015.15

Ashby, W. R. (1956). *Introduction to Cybernetics*. London, Chapman and Hall.

Baluška, F. and Levin, M. (2016). On having no head: Cognition throughout biological systems. *Front. Psych*. 7, 902. https://doi.org/10.3389/fpsyg.2016.00902

Barabási, A. L. and Albert, R. (1999). Emergence of scaling in random networks. *Science* 286: 509-512. https://doi.org/10.1126/science.286.5439.509

Barabási, A. L. and Oltvai, Z. N. (2004). Network biology: Understanding the cell's functional organization. *Nat. Rev. Genet*. 5: 101-114. https://doi.org/10.1038/nrg1272

Bassett, D. S. and Bullmore, E. (2006). Small world brain networks. *Neuroscientist* 12: 512-523. https://doi.org/10.1177/1073858406293182

Bauer R. and Kaiser M. (2017). Organisational principles of connectomes: Changes during evolution and development. In: Shigeno, S., Murakami, Y. and Nomura, T. (Eds) Brain Evolution by Design. Diversity and Commonality in Animals. Springer, Tokyo (pp. 387-401). https://doi.org/10.1007/978-4-431-56469-0_17

Beisson, J. and Sonneborn, T. M. (1965). Cytoplasmic inheritance of the organization of the cell cortex in *Paramecium aurelia. Proc. Natl. Acad. Sci. USA* 53(2), 275-282. https://doi.org/10.1073/pnas.53.2.275

Boilly, B., Faulkner, S., Jobling, P. and Hondermarck, H. (2017). Nerve dependence: From regeneration to cancer. *Cancer Cell* 31, 342-354. http://dx.doi.org/10.1016/j.ccell.2017.02.005

Botting, J. P. and Muir, L. A. (2018). Early sponge evolution: A review and phylogenetic framework. *Paleoworld* 27, 1-29. https://doi.org/10.1016/j.palwor.2017.07.001

Brown, J. B. et al. (2014). Diversity and dynamics of the *Drosophila* transcriptome. *Nature* 512, 393-399. https://doi.org/10.1038/nature12962

Brunet, T. and King, N. (2017). The origin of animal multicellularity and cell differentiation. *Devel. Cell* 43, 124-40. https://doi.org/10.1016/j.devcel.2017.09.016

Bucher, D. and Anderson, P. A. V. (2015). Evolution of the first nervous systems – what can we surmise? *J. Expt. Biol.* 218, 501-503. https://doi.org/10.1242/jeb.111799

Burkhardt, P. and Sprecher, S. G. (2017). Evolutionary origin of synapses and neurons – Bridging the gap. *BioEssays* 39, 1700024. https://doi.org/10.1002/bies.201700024

Cannon, J. T., Vellutini, B. C., Smith III, J., Ronquist, F., Jondelius, U. and Hejnol, A. (2016). Xenacoelomorpha is the sister group to Nephrozoa. *Nature* 530, 89-93. https://doi.org/10.1038/nature16520

Cebrià, F. (2008). Organization of the nervous system in the model planarian *Schmidtea mediterranea*: An immunocytochemical study. *Neurosci. Res*. 61, 375-384. https://doi.org/10.1016/j.neures.2008.04.005

Chernet, B. T., Fields, C. and Levin, M. (2015). Long-range gap junctional signaling controls

oncogene-mediated tumorigenesis in *Xenopus laevis* embryos. *Front. Physiol*. 5: 519. https://doi.org/10.3389/fphys.2014.00519

Chernet, B. T. and Levin, M. (2013). Endogenous voltage potentials and the microenvironment:
Bioelectric signals that reveal, induce and normalize cancer. *J. Clin. Exp. Oncol.* 2013, S1. http://dx.doi.org/10.4172/2324-9110.S1-002

Conant, R. C. and Ashby, W. R. (1970). Every good regulator of a system must be a model of that system. *Int. J. Systems Sci.* 1: 89-97. https://doi.org/10.1080/00207727008920220

Csaba, G. (2012). The hormonal system of the unicellular *Tetrahymena*: A review with evolutionary
aspects. *Acta Microbiol. Immunol. Hungarica* 59, 131-156. https://doi.org/10.1556/AMicr.59.2012.2.1

Dahal, G. R., Rawson, J., Gassaway, B., Kwok, B., Tong, Y., Ptácek, L. J. and Bates, E. (2012). An inwardly rectifying K+ channel is required for patterning. *Development* 139, 3653-3664. https://doi.org/10.1242/dev.078592

Darroch, S. A. F., Smith, E. F., Laflamme, M. and Erwin, D. H. (2018). Ediacaran extinction and Cambrian explosion. *Trends Ecol. Evol.* 33, 653-662. https://doi.org/10.1016/j.tree.2018.06.003

de Wiljes, O.O., van Elburg, R.A., Biehl, M. and Keijzer, F.A. (2015). Modeling spontaneous activity across an excitable epithelium: Support for a coordination scenario of early neural evoluti*on. Front. Comput. Neuro*sci. 9, 110. https://doi.org/10.3389/fncom.2015.00110

de Wiljes, O.O., van Elburg, R.A.J. and Keijzer, F.A. (2017). Modelling the effects of short and random proto-neural elongations. *J. R. Soc. Interface* 14, 20170399. https://doi.org/10.1098/rsif.2017.0399

de la Monte, S. M. and Kril, J. J. (2014). Human alcohol-related neuropathology. *Acta Neuropath.* 127, 71-90. https://doi.org/10.1007/s00401-013-1233-3

Dohrmann, M. and Wörheide, G. (2017). Dating early animal evolution using phylogenomic data. *Nat. Sci. Rep.* 7: 3599. https://doi.org/10.1038/s41598-017-03791-w

DuBuc, T. Q., Ryan, J. and Martindale, M. Q. (2019). "Dorsal-ventral" genes are part of an ancient axial patterning system: Evidence from *Trichoplax adhaerens* (Placozoa). *Mol. Biol. Evol.* in press. https://doi.org/10.1093/molbev/msz025

Durant, F., Bischof, J., Fields, C., Morokuma, J., LaPalme, J., Hoi, A. and Levin, M. (2019). The role of early bioelectric signals in the regeneration of planarian anterior-posterior polarity. *Biophys. J.* 116, in press. https://doi.org/10.1016/j.bpj.2019.01.029

Elliott, S. A. and Sánchez Alvarado, A. (2012). The history and enduring contributions of planarians
to the study of animal regeneration. *WIRES Dev. Biol*. 2, 301-326. https://doi.org/10.1002/wdev.82

Erwin, D. H. (2015). Early metazoan life: Divergence, environment and ecology. *Phil. Trans. R. Soc. B* 370: 20150036. http://dx.doi.org/10.1098/rstb.2015.0036

Farkas, J. E. and Monaghan, J. R. (2017). A brief history of the study of nerve dependent regeneration. *Neurogenesis* 4, e1302216. https://doi.org/10.1080/23262133.2017.1302216

Feuda, R., Dohrmann, M., Pett, W. et al., (2017). Improved modeling of compositional heterogeneity
supports sponges as sister to all other animals. *Curr. Biol.* 37: 3864-3870. https://doi.org/10.1016/j.cub.2017.11.008

Fields, C. and Glazebrook, J. F. (2017). Disrupted development and imbalanced function in the global neuronal workspace: A positive-feedback mechanism for the emergence of ASD in early infancy. *Cogn. Neurodyn*. 11: 1-21. https://doi.org/10.1007/s11571-016-9419-8

Fields, C. and Levin, M. (2018). Multiscale memory and bioelectric error correction in the cytoplasm-cytoskeleton-membrane system. *WIRES Syst. Biol. Med.* 10: e1410. https://doi.org/10.1002/wsbm.1410

Fields, C. and Levin, M. (2019). Somatic multicellularity as a satisficing solution to the prediction-error minimization problem. *Commun. Integr. Biol.* 12, 119-132. https://doi.org/10.1080/19420889.2019.1643666

Fischer, A. H. L., Pang, K., Henry, J. Q. and Martindale, M. Q. (2014). A cleavage clock regulates features of lineage-specific differentiation in the development of a basal branching metazoan, the ctenophore *Mnemiopsis leidyi*. *EvoDevo* 5, 4. https://doi.org/10.1186/2041-9139-5-4

Friston, K. (2013). Life as we know it. *J. R. Soc. Interface* 10: 20130475. http://dx.doi.org/10.1098/rsif.2013.0475

Friston, K, Levin, M., Sengupta, B. and Pezzulo, G. (2015). Knowing one's

place: A free-energy approach to pattern regulation. *J. R. Soc. Interface* 12, 20141383. http://dx.doi.org/10.1098/rsif.2014.1383

Funayama, N. (2013). The stem cell system in demosponges: Suggested involvement of two types of cells: archeocytes (active stem cells) and choanocytes (food-entrapping flagellated cells). *Devel. Genes Evol*. 223, 23-38. https://doi.org/10.1007/s00427-012-0417-5

Galliot, B. and Quiquand, M. (2011). A two-step process in the emergence of neurogenesis. *Europ. J. Neurosci.* 34, 847-862. https://doi.org/10.1111/j.1460-9568.2011.07829.x

Gavilán, B., Perea-Atienza, E. and Martínez, P. (2016). Xenacoelomorpha: A case of independent
nervous system centralization? *Phil. Trans. R. Soc. B* 371: 20150039. http://dx.doi.org/10.1098/rstb.2015.0039

Gerber, S. (2017). The geometry of morphospaces: lessons from the classic Raup shell coiling model. *Biol. Rev. Camb. Phil. Soc*. 92, 1142-1155. https://doi.org/10.1111/brv.12276

Hartenstein, V. and Stollewerk, A. (2015). The evolution of early neurogenesis. *Devel. Cell* 32, 390-407. http://dx.doi.org/10.1016/j.devcel.2015.02.004

Harwood, A. J. (2009). Dictyostelium Development: A Prototypic Wnt Pathway? Wnt Signaling. E. Vincan. Totowa, NJ, Humana Press**:** 21-32.

Herrera-Rincon, C. and Levin, M. (2018). Booting up the organism during development: Pre-behavioral functions of the vertebrate brain in guiding

body morphogenesis. *Commun. Integr. Biol.* 11, e1433440. https://doi.org/10.1080/19420889.2018.1433440

Herrera-Rincon, C., Pai, V.P., Moran, K.M., Lemire, J.M. and Levin, M. (2017). The brain is required for normal muscle and nerve patterning during early *Xenopus* development. *Nat. Commun.* 8, 587. https://doi.org/10.1038/s41467-017-00597-2

Hirth, F. (2010). On the origin and evolution of the tripartite brain. *Brain Behav. Evol.* 76, 3-10. https://doi.org/10.1159/000320218

Holland, L. Z. (2009). Chordate roots of the vertebrate nervous system: Expanding the molecular toolkit. *Nat. Rev. Neurosci*. 10, 736-746. https://doi.org/10.1038/nrn2703

Holland, L. Z. et al. (2014). Evolution of bilaterian central nervous systems: A single origin? EvoDevo 4, 27. https://doi.org/10.1186/2041-9139-4-27

Holstein, T. W. (2012). The evolution of the Wnt pathway. *Cold Spring Harbor Perspect. Biol.* 4,
a007922. https://doi.org/10.1101/cshperspect.a007922

Jékely, G., Keijzer, F. and Godfrey-Smith, P. (2015). An option space for early neural evolution. *Phil. Trans. R. Soc. Lond. B: Biol. Sci.* 370, 20150181. https://doi.org/10.1098/rstb.2015.0181

Kaiser, M. and Varier, S. (2011). Evolution and development of brain networks: From *Caenorhabditis elegans* to *Homo sapiens. Network: Comput. Neural Syst.* 22, 143-147. https://doi.org/10.3109/0954898X.2011.638968

Kamm, R. D. et al. (2018). Perspective: The promise of multi-cellular engineered living systems. *APL Bioeng*. 2, 040901 . https://doi.org/10.1063/1.5038337

Kandel, E. R., Schwartz, J. H. and Jessell, T. M. (1981). *Principles of Neural Science*. Amsterdam: Elsevier.

Kang, J. K. et al. (2011). Spatiotemporal transcriptome of the human brain. *Nature* 478, 483-489. https://doi.org/10.1038/nature10523

Keijzer, F. (2015). Moving and sensing without input and output: early nervous systems and the origins of the animal sensorimotor organization. *Biol. Philos*. 30, 311-331. https://doi.org/10.1007/s10539-015-9483-1

Keijzer, F. and Arnellos, A. (2017). The animal sensorimotor organization: a challenge for the environmental complexity thesis. *Biol. Philos*. 32, 421-441. https://doi.org/10.1007/s10539-017-9565-3

Keijzer, F., van Duijn, M. and Lyon, P. (2013). What nervous systems do: Early evolution, input-output, and the skin brain thesis. *Adapt. Behav*. 21, 67-85. https://doi.org/10.1177/1059712312465330

Kelava, I., Rentzsch, F. and Technau, U. (2015). Evolution of eumetazoan nervous systems: Insights from cnidarians. *Phil. Trans. R. Soc. B* 370, 20150065. http://dx.doi.org/10.1098/rstb.2015.0065

Koizumi, O. (2002). Developmental neurobiology of hydra, a model animal of cnidarians. *Can. J. Zool.* 80, 1678-1689. https://doi.org/10.1139/Z02-134

King, R. S. and Newmark, P. A. (2012). The cell biology of regeneration. J. Cell Biol. 196, 553-562. https://doi.org/10.1083/jcb.201105099

Kuchling, F., Friston, K., Georgiev, G. and Levin, M. (2019). Morphogenesis as Bayesian inference: A variational approach to pattern formation and control in complex biological systems. *Phys. Life Rev.* in press. https://doi.org/10.1016/j.plrev.2019.06.001

Kumar, A. and Brockes, J.P. (2012). Nerve dependence in tissue, organ, and appendage regeneration. *Trends Neurosci*. 35, 691-699. https://doi.org/10.1016/j.tins.2012.08.003

Kuol, N., Stojanovska, L., Apostolopoulos, V. and Nurgali, K. (2018). Role of the nervous system in cancer metastasis. *J. Expt. Clin. Cancer Res.* 37, 5. https://doi.org/10.1186/s13046-018-0674-x

Kyurkchiev, D., Bochev, I., Ivanova-Todorova, E., Mourdjeva, M., Oreshkova, T., Belemezova, K. and Kyurkchiev, S. (2014). Secretion of immunoregulatory cytokines by mesenchymal stem cells. *World J. Stem Cells* 26, 552-570. https://doi.org/10.4252/wjsc.v6.i5.552

Latora, V. and Marchiori, M. (2001). Efficient behavior of small-world networks. *Phys. Rev. Lett.* 87, 198701. https://doi.org/10.1103/PhysRevLett.87.198701

Lenard, J. (1992). Mammalian hormones in microbial cells. *Trends Biochem. Sci.* 17, 147-50. https://doi.org/10.1016/0968-0004(92)90323-2

Levin, M. (2006). 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*. (Part C) 78, 191-223. https://doi.org/10.1002/bdrc.20078

Levin, M. (2011). The wisdom of the body: Future techniques and approaches to morphogenetic fields in regenerative medicine, developmental biology and cancer. *Regen. Med.* 6: 667-673. https://doi.org/10.2217/RME.11.69

Levin, M. (2012). Morphogenetic fields in embryogenesis, regeneration, and cancer: non-local control of complex patterning. *Biosystems* 109, 243-261. https://doi.org/10.1016/j.biosystems.2012.04.005

Levin M. (2014). Molecular bioelectricity: How endogenous voltage potentials control cell behavior and instruct pattern regulation in vivo. *Mol. Biol. Cell.* 25: 3835-3850. https://doi.org/10.1091/mbc.e13-12-0708

Levin, M., Buznikov, G. A. and Lauder, J. M. (2006). Of minds and embryos: Left-right asymmetry and the serotonergic controls of pre-neural morphogenesis. *Devel. Neurosci*. 28, 171-185. https://doi.org/10.1159/000091915

Levin, M. and Martyniuk, C. J. (2018). The bioelectric code: An ancient computational medium for dynamic control of growth and form. *BioSystems* 164, 76-93. https://doi.org/10.1016/j.biosystems.2017.08.009

Leys, S. P. (1999). The choanosome of hexactinellid sponges. *Invert. Biol.* 118, 221-235. https://doi.org/10.2307/3226994

Leys, S. P. (2015). Elements of a `nervous system' in sponges. *J. Expt. Biol.* 218, 581-591. https://doi.org/10.1242/jeb.110817

Li, M., Duncan, K., Topp, C. N. and Chitwood, D. H. (2017). Persistent homology and the branching topologies of plants. *Am. J. Botany* 104: 349-353. https://doi.org/10.3732/ajb.1700046

Liebeskind, B. J., Hofmann, H. A., Hillis, D. M. and Zakon, H. H. (2017). Evolution of animals neural systems. *Annu. Rev. Ecol. Evol. Syst.* 48, 377-398. https://doi.org/10.1146/annurev-ecolsys-110316-023048

Liu, J., Martinez-Corral, R., Prindle, A., Lee, D. D., Larkin, J., Gabalda-Segarra, M., Garcia-Ojalvo, J. and Süel, G. M. (2017). Coupling between distant biofilms and emergence of nutrient time-sharing. *Science* 356, 638-642. https://doi.org/10.1126/science.aah4204

Loh, K. M., van Amerongen, R. and Nuss, R. (2016). Generating cellular diversity and spatial form: Wnt signaling and the evolution of multicellular animals. *Devel. Cell* 38, 643-655. http://dx.doi.org/10.1016/j.devcel.2016.08.011

Lutz-Meindl, U. (2016). *Micrasterias* as a model system in plant cell biology. *Front. Plant Sci.* 7: 999. https://doi.org/10.3389/fpls.2016.00999

Machemer, H. and Eckert, R. (1973). Electrophysiological control of reversed ciliary beating in *Paramecia.* *J. Gen. Physiol*. 61, 572-587. https://doi.org/10.1085/jgp.61.5.572

Manicka, S. and Levin, M. (2019). The Cognitive Lens: a primer on conceptual tools for analysing information processing in developmental and regenerative morphogenesis. *Phil. Trans. R. Soc. B*, 374: 20180369. https://doi.org/10.1098/rstb.2018.0369

Martín-Durán, J. M., Monjo, F. and Romero, R. (2012). Planarian embryology in the era of comparative developmental biology. *Int. J. Dev. Biol.* 56, 39-48. https://doi.org/10.1387/ijdb.113442jm

Martínez, P., Perea-Atienza, E., Gavilán, B., Fernandez, C. and Sprecher, S. (2017). The study of xenacoelomorph nervous systems: Molecular and morphological perspectives. *Invert. Zool*. 14, 32-44. https://doi.org/10.15298/invertzool.14.1.06

Masotti, A., Uva, P., Davis-Keppen, L., Basel-Vanagaite, L., Cohen, L. et al. (2015). Keppen-Lubinsky syndrome is caused by mutations in the inwardly-rectifying K+ channel encoded by KCNJ6. *Am. J. Human Genet.* 96, 295-300. https://doi.org/10.1016/j.ajhg.2014.12.011

Matthews, J. and Levin, M. (2018). The body electric 2.0: Recent advances in developmental bioelectricity for regenerative and synthetic bioengineering. *Curr. Opin. Biotech*. 52, 134-144. https://doi.org/10.1016/j.copbio.2018.03.008

McLaughlin, K. A. and Levin, M. (2018). Bioelectric signaling in regeneration: Mechanisms of ionic controls of growth and form. *Dev. Biol*. 433, 177-189. https://doi.org/10.1016/j.ydbio.2017.08.032

Meindl, U. (1993). *Micrasterias* cells as a model system for research on morphogenesis. *Microbiol. Rev.* 57: 415-433.

Modzelewska, K., Lauritsen, A., Hasenoeder, S., Brown, L., Georgiou, J. and Moghal, N. (2013). Neurons refine the *Caenorhabditis elegans* body plan by directing axial patterning by Wnts. *PloS Biol.* 11, e1001465. https://doi.org/10.1371/journal.pbio.1001465

Mondia, J.P., Levin, M., Omenetto, F.G., Orendorff, R.D., Branch, M.R. and Adams, D.S. (2011). Long-distance signals are required for morphogenesis of the regenerating *Xenopus* tadpole tail, as shown by femtosecond-laser ablation. *PloS One* 6, e24953. https://doi.org/10.1371/journal.pone.0024953

Moore, D., Walker, S. I. And Levin, M. (2017). Cancer as a disorder of patterning information: computational and biophysical perspectives on the cancer problem. *Converg, Sci. Phys. Oncol.* 3, 043001. https://doi.org/10.1088/2057-1739/aa8548

Moreno-Smith et al. (2011). Dopamine blocks stress-mediated ovarian carcinoma growth. Clin. Cancer Res. 17, 3649-3659. https://doi.org/10.1158/1078-0432.CCR-10-2441

Moroz, L. L. (2015a). Convergent evolution of neural systems in ctenophores. *J. Expt. Biol.* 218, 598-611. https://doi.org/10.1242/jeb.110692

Moroz, L. L. and Kohn, A. B. (2015). Independent origins of neurons and synapses: Insights from ctenophores. *Phil. Trans. R. Soc. B* 371, 20150041. http://dx.doi.org/10.1098/rstb.2015.0041

Moroz, L. L. et al. (2006). Neuronal transcriptome of *Aplysia*: Neuronal compartments and circuitry. *Cell* 127, 1453-1467. https://doi.org/10.1016/j.cell.2006.09.052

Morris, H. R., Taylor, G. W., Masento, M. S., Jermyn, K. A. and Kay, R. R. (1987). Chemical structure of the morphogen differentiation inducing factor from *Dictyostelium discoideum. Nature* 328: 811-814. https://doi.org/10.1038/328811a0

Morris, H. R., Masento, M. S., Taylor, G. W., Jermyn, K. A. and Kay, R. R. (1988). Structure elucidation of two differentiation inducing factors (DIF-2 and DIF-3) from the cellular slime mould *Dictyostelium discoideum. Biochem. J.* 249: 903-906. https://doi.org/10.1042/bj2490903

Narbonne, G. M., Xiao, S and Shields, G. A. (2012). The Ediacaran Period. In: Gradstein, F. M., Ogg, J. G., Schmitz, M. and Ogg, G. (Eds.) *The Geologic Time Scale*. Amsterdam: Elsevier (pp. 413-435). https://doi.org/10.1016/B978-0-444-59425-9.00018-4

Nelsen, E. M., Frankel, J. and Jenkins, L. M. (1989). Non-genic inheritance of cellular handedness. *Development* 105(3), 447-456. PMID: 2612360

Neville, A. C. (1976). *Animal Asymmetry*. London: Edward Arnold.

Newman, M. E. J. (2001). The structure of scientific collaboration networks. *Proc. Natl. Acad. Sci. USA* 98: 404-409. https://doi.org/10.1073/pnas.98.2.404

Nickel, M. (2010). Evolutionary emergence of synaptic nervous systems: What can we learn from the non-synaptic, nerveless Porifera? *Invert. Biol.* 129, 1-16. https://doi.org/10.1111/j.1744-7410.2010.00193.x

Nielsen, C. (2008). Six major steps in animal evolution: Are we derived sponge larvae? Evol. Devel. 10, 241-257. https://doi.org/10.1111/j.1525-142X.2008.00231.x

Northcutt, R. G. (2002). Understanding vertebrate brain evolution. *Integ. Comp. Biol*. 42, 743-756. https://doi.org/10.1093/icb/42.4.743

Northcutt, R. G. (2012). Evolution of centralized nervous systems: Two schools of evolutionary thought. *Proc. Natl. Acad. Sci. USA* 109, 10626-10633. https://doi.org/10.1073/pnas.1201889109

Nutman, A. P., Bennett, V. C., Friend, C. R. L., Van Kranendonk, M. J. and Chivas, A. (2016). Rapid emergence of life shown by discovery of 3,700-

million-year-old microbial structures. *Nature* 537: 535-538. https://doi.org/10.1038/nature19355

Ozgen, H., Hellemann, G. S., Stellato, R. K., Lahuis, B., van Daalen, E., Staal, W. G., Rozendal, M., Hennekam, R. C., Beemer, F. A. and van Engeland, H. (2011). Morphological features in children with Autism Spectrum Disorders: A matched case–control study. *J. Autism Devel. Disord*. 41, 23-31. https://doi.org/10.1007/s10803-010-1018-7

Pagán, O. R. (2014). *The first brain: The neuroscience of planarians*. Oxford: Oxford University Press.

Palmer, A. R. (2009). Animal asymmetry. Curr. Biol. 19, R473-R477. https://doi.org/10.1016/j.cub.2009.04.006

Pattee, H. H. (2001). The physics of symbols: Bridging the epistemic cut. *Biosystems* 60, 5-21. https://doi.org/10.1016/S0303-2647(01)00104-6

Pawlowski, A. and Weddell, G. (1967). Induction of tumors in debervated skin. *Nature* 213, 1234-1236. https://doi.org/10.1038/2131234a0

Pendleton, J. W., Nagai, B. K., Murtha, M. T. and Ruddle, F. H. (1993). Expansion of the Hox gene family and the evolution of chordates. *Proc. Natl. Acad. Sci. USA* 90, 6300-6304. https://doi.org/10.1073/pnas.90.13.6300

Penfield, W., & Boldrey, E. (1937). Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. Brain 60, 389-443. https://doi.org/10.1093/brain/60.4.389

Pezzulo G, Levin M. (2015). Re-membering the body: Applications of computational neuroscience to the top-down control of regeneration of limbs

and other complex organs. *Integr. Biol. (Camb.)* 7: 1487-1517. https://doi.org/10.1039/C5IB00221D

Pezzulo G, Levin M. (2016). Top-down models in biology: Explanation and control of complex living systems above the molecular level. *J. R. Soc. Interface* 13: 20160555. https://doi.org/10.1098/rsif.2016.0555

Pietak, A., Bischof, J., LaPalme, J., Morokuma, J. and Levin, M. (2019). Neural control of body-plan axis in regenerating planaria. *PLoS Comput. Biol*. 15, e1006904. https://doi.org/10.1371/journal.pcbi.1006904

Pietak, A. and Levin, M. (2017). Bioelectric gene and reaction networks: computational modelling of genetic, biochemical and bioelectrical dynamics in pattern regulation. *J. R. Soc. Interface* 2017, 20170425. https://doi.org/10.1098/rsif.2017.0425

Piraino, S., Zega, G., Benedetto, A., Dell'Anna, A., Pennati, R., Carnevali, D. C., Schmid, V. and Reichert, H. (2011). Complex neural architecture in the diploblastic larva of *Clava multicornis* (Hydrozoa, Cnidaria). *J. Comp. Neurol.* 519, 1931-1951. https://doi.org/10.1002/cne.22614

Plattner, H. and Verkhratsky, A. (2018). The remembrance of the things past: Conserved signalling pathways link protozoa to mammalian nervous system. Cell Calcium 73, 25-39.
https://doi.org/10.1016/j.ceca.2018.04.001

Polanyi, M. (1968). Life's irreducible structure. Live mechanisms and information in DNA are boundary conditions with a sequence of boundaries above them. *Science* 160, 1308-1312. https://doi.org/10.1126/science.160.3834.1308

Poretti, A.,Meoded, A., Ceritoglu, E., Boltshauser, E. and Hulsman, T. A. G. M. (2010). Postnatal in-vivo MRI findings in anencephaly. *Neuropediatrics* 41, 264-266. http://dx.doi.org/10.1055/s-0031-1271658

Prieto-Barajas, C. M., Valencia-Cantero, E. and Santoyo, G. (2018). Microbial mat ecosystems: Structure types, functional diversity, and biotechnological application. *Elect. J. Biotech*. 31: 48-56. https://doi.org/10.1016/j.ejbt.2017.11.001

Prindle, A., Liu, J., Asally, M., Ly, S., Garcia-Ojalvo, J. and Süel, G. M. (2015). Ion channels enable electrical communication in bacterial communities. *Nature* 527, 59-63. https://doi.org/10.1038/nature15709

Prusinkiewicz, P. (1998). Modeling of spatial structure and development of plants. *Scientia Horticult.* 74: 113-149. https://doi.org/10.1016/S0304-4238(98)00084-3

Ramachandra, N. B., Gates, R. D., Ladurner, P., Jacobs, D. K. and Hartenstein, V. (2002). Embryonic development in the primitive bilaterian *Neochildia fusca*: Normal morphogenesis and isolation of POU genes *Brn-1 and Brn-3. Devel. Genes Evol.* 212, 55-69. https://doi.org/10.1007/s00427-001-0207-y

Rosen, R. (1986). On information and complexity. In: Casti, J. L., Karlqvist, A. (Eds.) *Complexity, Language, and Life: Mathematical Approaches*. Berlin: Springer-Verlag. (pp. 174-196).

Roshchina, V. V. (2016). New trends and perspectives in the evolution of neurotransmitters in microbial, plant, and animal cells. In: Lyte, M. (Ed.) *Microbial Endocrinology: Interkingdom Signaling in Infectious Disease and Health*. Cham, Switzerland: Springer (pp. 25-77). https://doi.org/10.1007/978-3-319-20215-0_2

Schaller, H. C., Hermans-Borgmeyer, I. and Hoffmeister, S. A. H. (1996). Neuronal control of development in hydra. *Int. J. Dev. Biol.* 40, 339-344. PMID: 8735946

Sebé-Pedrós, A., Degnan, B. M. and Ruiz-Rillo, I. (2017). The origin of Metazoa: A unicellular perspective. *Nat. Rev. Genet.* 18: 498-512. https://doi.org/10.1038/nrg.2017.21

Simion, P., Philippe, H., Baurain, D., et al. (2017). A large and consistent phylogenomic dataset supports sponges as the sister group to all other animals. *Curr. Biol.* 27: 958-967. http://dx.doi.org/10.1016/j.cub.2017.02.031

Smetana, K. Jr., Dvořánková, B. and Lacina, L. (2013). Phylogeny, regeneration, ageing and cancer: Role of microenvironment and possibility of its therapeutic manipulation. *Folia Biol*. (Praha) 59, 207-216. PMID:24485302

Sogabe, S., Hatleberg, W. L., Kocot, K. M., Say, T. E., Stoupin, D., Roper, K. E., Fernandez-Valverde, S. L., Degnan, S. M. and Degnan, B. M. (2019). Pluripotency and the origin of animal multicellularity. *Nature* in press. https://doi.org/10.1038/s41586-019-1290-4

Sporns, O. and Honey, C. J. (2006). Small worlds inside big brains. *Proc. Natl. Acad. Sci. USA* 111: 15220-15225. https://doi.org/10.1073/pnas.0609523103

Stal, L. J. (2012). Cyanobacterial mats and stromatolites. In: Whitton, B. A. (Ed.), *Ecology of Cyanobacteria II: Their Diversity in Space and Time*. Berlin: Springer (pp. 65-125).

https://doi.org/10.1007/978-94-007-3855-3_4

Stone, J. R. (1997). The spirit of D'arcy Thompson dwells in empirical morphospace. Math. Biosci. 142, 13-30. PMID: 9125858

Sullivan, K. G., Emmons-Bell, M. and Levin, M. (2016). Physiological inputs regulate species-specific anatomy during embryogenesis and regeneration. *Commun. Integr. Biol*. 9, e1192733. https://doi.org/10.1080/19420889.2016.1192733

Tepass, U., Truong, K., Godt, D., Ikura, M. and Peifer, M. (2000). Cadherins in embryonic and neural morphogenesis. Nat. Rev. Mol. Cell Biol. 1, 91-100. https://doi.org/10.1038/35040042

Tran, C. and Damaser, M. S. (2015). Stem cells as drug delivery methods: Application of stem cell secretome for regeneration. *Adv. Drug Deliv. Rev*. 82-83, 1-11. https://doi.org/10.1016/j.addr.2014.10.007

Tristani-Firouzi, M. and Etheridge, S. P. (2010). Kir 2.1 channelopathies: the Andersen-Tawil syndrome. *Pflugers Arch.* 460, 289-294. https://doi.org/10.1007/s00424-010-0820-6

Tseng A, Levin M. (2013). Cracking the bioelectric code: Probing endogenous ionic controls of pattern formation. *Commun. Integr. Biol.* 6: e22595. https://doi.org/10.4161/cib.22595

Turner, P. and Nottale, L. (2017). The physical principles underpinning self-organization in plants. *Prog. Biophys. Mol. Biol.* 123: 48-73. https://doi.org/10.1016/j.pbiomolbio.2016.09.003

Tweedt, S. M. and Erwin, D. H. (2015). Origin of metazoan developmental toolkits and their expression in the fossil record. In: Ruiz-Trillo, I. and Nedelcu, A. M. (Eds.) *Evolutionary Transitions to Multicellular Life*. Dordrecht: Springer (pp. 47-78). https://doi.org/10.1007/978-94-017-9642-2_3

Usui-Ishihara, A., Simpson, P. and Usui, K. (2000). Larval multidendrite neurons survive metamorphosis and participate in the formation of imaginal sensory axonal pathways in the Notum of Drosophila. *Devel. Biol.* 225, 357-369. https://doi.org/10.1006/dbio.2000.9838

Vandenberg, L. N., Adams, D. S. and Levin, M. (2012). Normalized shape and location of perturbed craniofacial structures in the Xenopus tadpole reveal an innate ability to achieve correct morphology. *Dev. Dyn*. 241, 863-878. https://doi.org/10.1002/dvdy.23770

Vandenberg, L. N., Lemire, J. M. and Levin, M. (2013). It's never too early to get it Right. *Commun. Integr. Biol.* 6, e27155. http://dx.doi.org/10.4161/cib.27155

Wallingford, J. B. and Mitchell, B. (2011). Strange as it may seem: The many links between Wnt signaling, planar cell polarity, and cilia. Genes Devel. 25, 201-213. https://doi.org/10.1101/gad.2008011

Watanabe, H., Hoang, V. T., Mättner, R. and Holstein, T. W. (2009). Immortality and the base of multicellular life: Lessons from cnidarian stem cells. *Sem. Cell Devel. Biol.* 20, 1114-1125. https://doi.org/10.1016/j.semcdb.2009.09.008

Watts, D. J. and Strogatz, S. H. (1998). Collective dynamics of `small-world' networks. *Nature* 393: 440-442. https://doi.org/10.1038/30918

Wenger, Y., Buzgariu, W. and Galliot, B. (2015). Loss of neurogenesis in *Hydra* leads to compensatory regulation of neurogenic and neurotransmission genes in epithelial cells. *Phil. Trans. R. Soc. B* 371, 20150040. http://dx.doi.org/10.1098/rstb.2015.0040

Whelan, N. V., Kocot, K. M., Moroz, et al. (2017). Ctenophore relationships and their placement as the sister group to all other animals. *Nat. Evol. Ecol.* 1, 1737-1746. https://doi.org/10.1038/s41559-017-0331-3

Wiesel, T. N. and Hubel, D. H. (1963a). Effects of visual deprivation on morphology and physiology of cells in the cat's lateral geniculate body. *J. Neurophysiol.* 26, 978-993. https://doi.org/10.1152/jn.1963.26.6.978

Wiesel, T. N. and Hubel, D. H. (1963b). Single-cell responses in striate cortex of kittens deprived of vision in one eye. *J. Neurophysiol.* 26, 1003-1017. https://doi.org/10.1152/jn.1963.26.6.1003

Williams, D. W. and Shepherd, D. (2002). Persistent larval sensory neurones are required for the normal development of the adult sensory afferent projections in *Drosophila*. *Development* 129, 617-624. PMID: 11830563