Morphoceuticals: Perspectives for discovery of drugs targeting anatomical control mechanisms in regenerative medicine, cancer and aging

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Morphoceuticals are a new class of interventions that target the setpoints of anatomical homeostasis for efficient, modular control of growth and form. Here, we focus on a subclass: electroceuticals, which specifically target the cellular bioelectrical interface. Cellular collectives in all tissues form bioelectrical networks via ion channels and gap junctions that process morphogenetic information, controlling gene expression and allowing cell networks to adaptively and dynamically control growth and pattern formation. Recent progress in understanding this physiological control system, including predictive computational models, suggests that targeting bioelectrical interfaces can control embryogenesis and maintain shape against injury, senescence and tumorigenesis. We propose a roadmap for drug discovery focused on manipulating endogenous bioelectric signaling for regenerative medicine, cancer suppression and antiaging therapeutics.

Keywords: Biomedicine; Drug discovery; Morphogenesis

Introduction: Anatomical homeostasis – A central concept for biomedicine

One crucial aspect of cell biology is the ability of groups of cells to collectively reach specific, large-scale anatomical outcomes. From single cells (eggs) to complex metazoan bodies, such cell collectives cooperate toward creating complex forms and defending them against tumorigenesis, aging and injuries. Understanding the set of mechanisms that allow this is crucial for producing innovating new therapeutics for birth defects, regenerative repair and cancer – transformative biomedical therapeutics would be within reach if there were robust methods to communicate anatomical goal states to cell groups, coaxing them to build fresh organs and tissues as needed. What features of morphogenesis can be exploited to achieve this?

Importantly, although reliable and consistent, morphogenesis is not hardwired – it is plastic and dynamic (Figure 1). Cell collectives have ample resources to deal with a wide range of disruptors of their morphogenetic target states: the same anatomical configuration can be reached from different starting conditions and despite significant perturbations.[1] For example, regenerative organisms, like salamanders, can regrow a limb after amputation. The cells reproduce a normal limb and stop their activity when the precisely correct appendage is complete, regardless of the level of the original amputation. Deer regrow huge amounts of antler bone and innervation each year, and even human beings organisms can regenerate livers. Tadpoles with scrambled faces still make normal frogs[2] because their craniofacial organs rearrange as needed during metamorphosis. This property is also found in the regulative development of early mammalian embryos that can recover from massive perturbation such as complete (natural or artificial) bisection, producing two identical normal bodies. Although cellular genetic hardware changes on evolutionary timescales, its remarkable physiological and computational (software) properties enable cellular collectives to form versatile machines that solve a wide range of problems in real time. The emerging field at the intersection of diverse intel-

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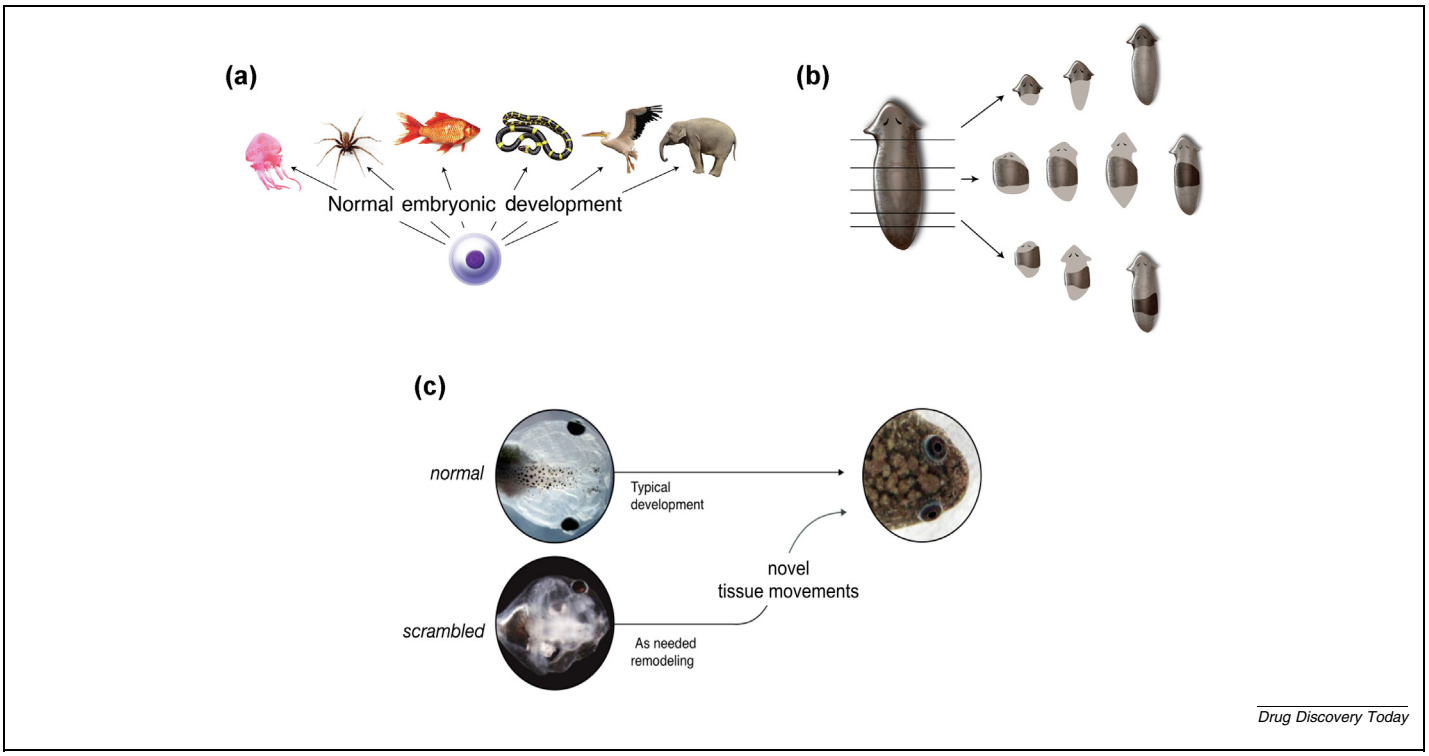

FIGURE 1

Morphogenesis from different starting conditions and despite significant perturbations in different organisms. **(a)** Normal embryonic development produces complex morphologies from the progeny of a single egg cell – in effect regenerating an entire body from one cell which reliably completes a complex path through morphospace to the correct target morphology. **(b)** Tiny pieces of planaria can rebuild the entire form of the organism by regeneration, rescaling as needed to maintain overall proportions. **(c)** The anatomical transition from tadpole to frog requires significant remodeling of the head. Highly abnormal tadpole faces still make normal frog heads as the different anatomical parts migrate and rearrange until a normal head target morphology is reached. Panels a and b by Jeremy Guay of Peregrine Creative. Panel c reproduced, with permission, from Ref. 17.

ligence, computer science and developmental biophysics is beginning to understand how cellular swarms deploy a kind of collective intelligence to navigate the space of possible anatomical shapes (morphospace) until the correct target morphology is reached, and despite perturbations.[3,4]

How do such cellular collectives know when to stop growing and remodeling? One set of models focuses on 'anatomical homeostasis' – the competency to implement an error-minimization scheme toward an anatomical setpoint.[4,5] This is a different way of thinking about morphogenesis – not as a set of outcomes that emerge inevitably from the unrolling of a fixed genome through molecular interactions but instead as a real-time problem-solving process carried out by a machine with context-specific information-processing capabilities. This difference in perspective is crucial because it has strong implications for therapeutic interventions.[6] Most current approaches in molecular medicine assume that the only viable therapeutic target is the hardware. Biological circuits must be rewired bottom-up (e.g., via genomic editing or drugs that target specific protein activities). By contrast, the homeostatic perspective suggests that it might be possible to work top-down, rewriting the anatomical target or setpoint to induce a different outcome without changing the morphogenetic machinery itself. This corresponds to the shift seen in information technology, which went from exclusively hardware-based control (in the 1940s and 1950s) to exploitation of reprogrammability, modularity of competent subroutines and high-level programming languages. Can medicine benefit from a similar transition?

Morphoceuticals are a novel class of therapeutics inspired by this perspective, developed to exploit the modular, error-correcting capabilities of tissues instead of trying to micromanage pathways. These can be repurposed existing compounds or newly discovered ones. What makes them unique is not a particular chemical property but rather the fact that they are used to target high-level morphogenetic control machinery instead of bottom-up targeting of specific molecular-level phenotypes. Their discovery stems from efforts to understand collective decision-making of cell groups navigating morphogenetic, transcriptional, physiological and metabolic problem spaces[3]; as the computational mechanisms that assess and modify growth and form are discovered, these sensing, memory and implementation steps become druggable targets.

Here, we focus on a subset of these: electroceuticals, which specifically target bioelectric networks using ion channel drugs and other modalities (Figure 2, Table 1). Electroceutical approaches using direct external stimulation of peripheral nerves have been covered elsewhere[7–12]; these have shown promise but face the limitations where, although suitable to trigger action potentials, electrodes are not able to set up the standing (stable) patterns of cellular V_{mem} that are essential for organ-level bioelectric control. Here, we focus on drug approaches for manipulating the native bioelectric interface (ion channels and gap junctions)

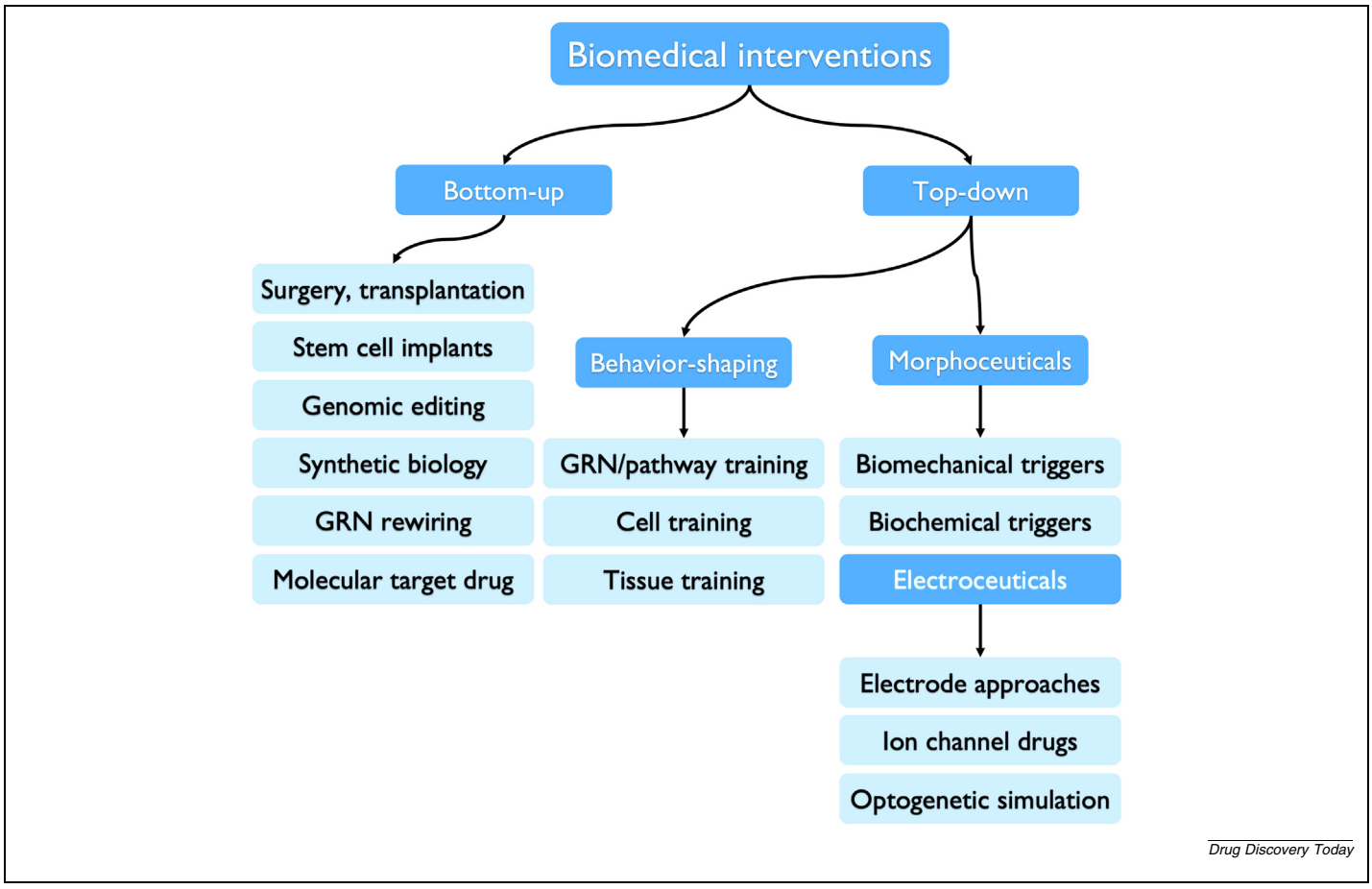

FIGURE 2

Landscape of biomedical interventions. Bottom-up approaches focus on micromanaging molecular mechanisms directly (rewiring the hardware) whereas top-down approaches seek to exploit the innate computational capacities of tissues by providing triggering stimuli (rewriting the software). Here, we focus on morphoceuticals and particularly on the subdomain of electroceuticals, which target ion channels (although other, non-bioelectric morphoceuticals, such as BMP- and WNT-pathway drugs that trigger complex anatomical outcomes, also exist). GRN = gene-regulatory network.

and especially expand its relevance beyond neurons to include all body cells, complementing exciting recent advances in using direct stimulation in regenerative contexts.[13–15] We review recent work that explains why bioelectric networks are such a compelling therapeutic target for developmental defects, regeneration and cancer. We also review evidence suggesting that aging, which has not yet been explored in the literature on bioelectricity,[16–18] constitutes another instantiation of morphogenetic dysregulation that could also respond to targeting bioelectric networks and offer our perspective on paths forward for electroceutical drug discovery.

Developmental bioelectricity: A medium for anatomical control

How do tissues store setpoints and coordinate cellular activity across distance[19]? Mostly occurring in the brain, this is implemented by bioelectrical networks[20] throughout the body. Developmental bioelectricity is the ubiquitous exchange of slowly changing ion-based voltage signals within and among cells,[21] which underlies collective decision-making at the tissue level. All cells respond to changes in transmembrane voltage potential (V_{mem}). In this sense, other cells are similar to neurons: their electric state is determined by their ion channels, transporters and pumps, and by gap junctions (electrical synapses), which propagate the electric state between neighboring cells (Figure 3a, b). The bioelectric state of a cell is a function of past experience, extracellular signaling and signals from immediate neighbors – those directly connected via gap junctions. Bioelectric signaling has been shown to regulate a range of phenotypes in human cells, for example differentiation in mesenchymal stem cells.[22–25]

More important than single cell states, however, is the fact that, together, the voltage state of cells form a network – a tissue-wide bioelectrical pattern that ultimately triggers the changes in gene expression required to attain a specific morphogenetic goal. The bioelectric code maps prepatterns of voltage into organ size, shape and placement information via transduction by a set of mechanisms including neurotransmitter gating, calcium signaling and voltage-sensitive phosphatases (Figure 3). Whereas the electrical neural networks of the brain guide muscles to implement behavioral goals, the ancient bioelectric networks of the body guide cell activity to implement morphogenetic goals, via storage and implementation of slowly changing anatomical prepatterns.[6,26–30]

TABLE 1

Sample morphoceuticals.

Drug	Molecular target	Large-scale phenotypic effect	Refs
SCH28080 (electroceutical)	H,K-ATPase exchanger pump	Control of bioelectric circuit specifying head–tail polarity → reverses two-headed animals back to normal 1-head form and reverses left–right asymmetry of organs	128,167
Ivermectin (electroceutical)	Glycine- and glutamate-gated chloride channels	Control of bioelectric properties of several tissues → induces melanoma phenotype, head shape change during regeneration and peripheral nerve regeneration	81,168,169
Monensin (electroceutical)	Sodium ionophore (depolarizer)	Control of bioelectric state guiding scarring and/or regeneration decision → induces tails to regenerate	63
Nigericin (electroceutical)	Potassium ionophore (depolarizer)	Control of bioelectric circuit specifying head–tail polarity → induces whole head formation	35
Lamotrigine, gabapentin (electroceutical)	HCN2 ion channel	Enhancing developmental compartments → repairs birth defects of face, heart and gut	170
Progesterone (biochemical)		Induces leg regeneration	65
MDT cocktail (BDNF, 1,4-DPCA, RD5, GH and RA) (biochemicals)	Variety of targets	Induces leg regeneration	64
Wnt activator + Shh + FGF10 + thymosin beta4 (biochemicals)	Variety of targets	Induces leg regeneration	171
Fluoxetine (biochemical)	Serotonin transporter inhibitor	Suppression of induced melanoma, reversal of asymmetry of body organs, increase of innervation and vision from ectopic eyes	80,152,172
Capsaicin (electroceutical)	TRPV1 agonist	Induction of axon outgrowth	173
Zolmitriptan (biochemical)	Serotonin receptor 1 agonist	Induces growth of ectopic nerve but not of endogenous normal nerve	152

Spatiotemporal patterns of V_{mem} *in vivo* are instructive for reaching specific morphogenetic outcomes. Microinjection of mRNA encoding Kir6.2 ion channels into blastomeres of frog embryos can reproduce the bioelectric state characteristic of the native eye field in ectopic locations[31] by mimicking the native patterns that regionalize the face.[32,33] Doing this in precursors of gut or other tissues – locations that never show competency to become eye tissue when induced by the 'master' eye gene Pax6 – induces the development of entire eyes that can connect to the nervous system and provide vision. Similarly, regenerating planaria, which normally reliably reproduce a head at one end and a tail at the other, can be coaxed into viable two-head or no-head forms by targeting the bioelectrical gradients in regenerating fragments,[34–36] or even to grow heads corresponding to other species of planaria,[37] all without any genomic editing. What these morphoceutical interventions have in common is that, in all cases, the input stimulus is informationally very simple – it does not attempt to provide instructions to individual cells on how to build an eye or a head, it provides a high-level master regulator that says 'build organ X here' and relies on the competency of the underlying cellular and molecular mechanisms to fill in the details.[1,3] This is a desirable property from the perspective of regenerative medicine interventions: the less we need to micromanage the more robust the outcomes. For example, inducing a second head in this manner produces well-scaled heads appropriate to the body, whereas induction using a canonical molecular mechanism (Wnt pathway) induces tiny heads because the size control has to be handled separately when approaching the problem bottom-up.[35]

As befits a native control system, ion channels have been implicated in numerous channelopathies of embryonic embryogenesis.[38–43] The human phenotypes are well-modeled by functional experiments in species like fruit fly, zebrafish, mouse and frog, implicating numerous ion channels such as Kir2.1 and gap junctions (connexins).[38,44–46]

Overall, the manipulation of the bioelectrical pattern holds great promise for the control of morphogenesis[47] and, thus, for regenerative medicine. Bioelectric interventions can work at several levels. For example, electrotaxis has been exploited for the control of cell movement in wound healing.[48–53] At the level of specific organs, malformations induced by mutations and teratogens can be reversed. For example, profound defects of brain structure and function induced by teratogens can be reversed by reinforcing normal endogenous voltage pre-patterns in xenopus.[54] Pai *et al.* showed that brain developmental defects induced by mutation of key neurogenesis genes such as Notch can be rescued by misexpression or activation of native HCN2 (hyperpolarization-activated cyclic nucleotide-gated) channels.[55] 56As voltage-regulated ion channels, HCN2 are context-sensitive: they increase hyperpolarization of slightly polarized cells but have no effect on depolarized cells. Thus, they function as 'contrast enhancers' that sharpen weakened differences in V_{mem} across compartment boundaries similar to the way a Sharpen filter works to emphasize order in a fuzzy image.

Pai *et al.* developed a computational model of the endogenous bioelectrical circuit that sets the brain prepattern, modeling the function of HCN2 channels to reinforce the voltage potential differences that demarcate the edges of the brain in development.[54]

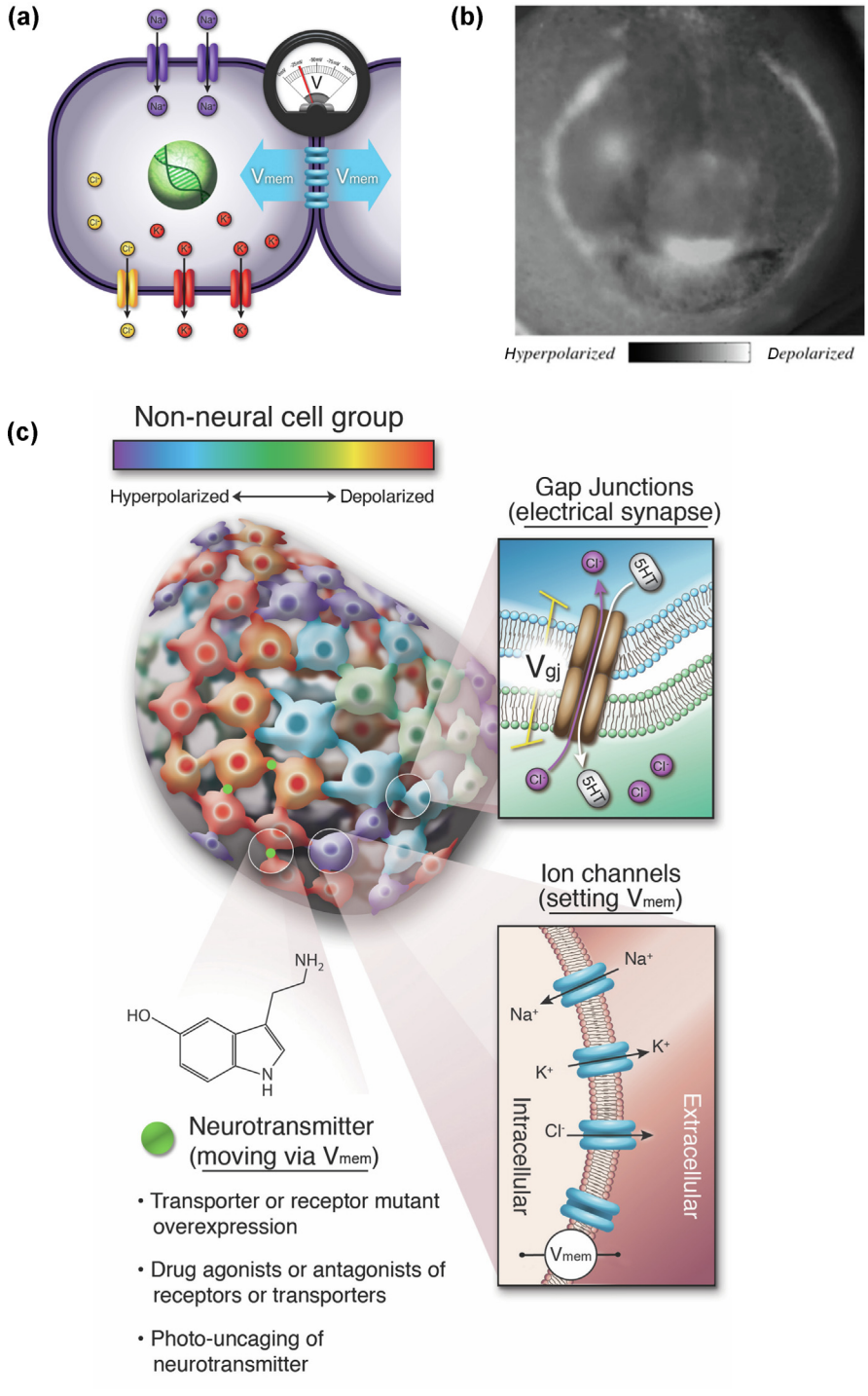

FIGURE 3

Mechanisms of developmental bioelectricity. **(a)** Movement of ions through ion channel proteins on all cell surfaces sets the V_{mem} (resting potential across the membrane). That V_{mem} can be communicated to the cell's neighbors via gap junctions (cyan arrows) – electric synapses. **(b)** The interactions of cell voltage transitions establish stable distributions of V_{mem} across tissues, such as shown here at one developmental timepoint of the frog embryo face, showing the future number and locations of eyes, mouth and other craniofacial organs. **(c)** Interventions designed to manipulate these gradients can target the native physiological computation machinery such as drug or light approaches to open or close ion channels and gap junctions, or move the neurotransmitter and other small molecular signals that propagate through these networks. Recent efforts have utilized computational models[58,174,175] to guide these techniques toward specific bioelectric patterning outcomes, which in turn regulate gene expression and cell movement to achieve organogenesis. Images a and c courtesy of Jeremy Guay of Peregrine Creative. Image b reproduced, with permission, from Ref. 32.

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This model suggested that bioelectric drugs that mimic the effect of HCN2 overexpression should rescue brain teratogenesis via local and long-range bioelectric repair. Indeed, lamotrigine and gabapentin, already approved for use in humans for other indications, rescued teratogen-induced defects in xenopus embryos.[56–58] The ability to repair complex organ defects of the brain, face, heart and gut by simple (and systemic) application of a single, repurposed channel activator reveals the potential of this approach – it relies on the computational capacity of the tissues to do the heavy lifting,[4,19] using computation to identify simple triggers that do not require manual control of gene levels, localization, stem cell behavior, among others.

Above the organ level, bioelectric signaling has been shown to be a promising target to induce the regeneration of entire appendages. In rats, limb regeneration can be augmented by bioelectric signals.[14,59–62] In a frog model of tail regeneration (which includes muscle, spinal cord, blood vessels and skin), complete regrowth in nonregenerative conditions has been shown by the 1-hour application of a simple ionophore, which induces a pro-regenerative state in the wound and then does not need to be maintained during the 10 days of regeneration.[63] The rapid inductive aspect is also seen in non-bioelectric morphoceuticals, such as a bioreactor-delivered drug cocktail in adult frogs that triggered 18+ months of leg regeneration[64,65] after just 24 h of treatment.

Cancer as a developmental defect: New diagnostic and treatment modalities

One way to think about cancer is as the shrinking of the computational boundary from organ-sized back down to the level of a single cell, at which point cells resume their ancient unicellular past and treat the rest of the body as external environment.[66–69] This model predicts that cancer would be driven by a dysregulation of the computational glue that normally keeps cells working toward organ-level morphogenetic goals,[70] for example bioelectric signaling.

It has been known for decades that tumors have depolarized bioelectric signatures and abnormal electrical connectivity compared with normal tissue,[71–74] in addition to excessive sodium accumulation.[75–77] Moreover, tumorigenesis starts with the bioelectric disconnection of cells from the larger somatic morphogenetic network[78,79]; and metastatic melanoma can be induced in the absence of carcinogens or oncogenic mutations simply by dysregulating the bioelectric signaling between two cell types.[80,81] Indeed, some authors have described cancer as a special case of channelopathies: the onco-channelopathies.[82–89] It is important to keep in mind that, whereas abnormal bioelectric states can be induced by mutant ion channels, the dynamic nature of channel signaling (they can be opened or closed post-translationally) ensures that a plethora of physiological events can induce these states in the absence of any genetic lesions.[90]

Studies in model organisms support the diagnostic and therapeutic promise of targeting the tumor bioelectric state (Figure 4). In tadpoles, nascent tumors induced by microinjection of human oncogene mRNA are depolarized compared with normal tissue and can be detected in advance using voltage-sensitive fluorescent dyes,[91] offering promise for early, noninvasive detection of cancer and for visualizing tumor boundaries during surgical intervention. Furthermore, co-injecting a hyperpolarizing ion channel greatly reduces tumor formation despite the overexpression of strong human oncogenes such as Xrel3 or KRAS.[91,92] Crucial for therapeutic potential, hyperpolarization can still produce cancer normalization when it occurs after tumors have formed, as well as at long-range, via a gap-junction-dependent mechanism.[93,94] This shows that hyperpolarization induced by pharmacological, genetic or even light-based stimuli could be used to prevent or revert tumors, and ion channels are beginning to be implicated in tumor resistance.[95] Because of their essential role in controlling the spread of morphogenetic (or cancer-suppressive) signals throughout the body, gap junctions and connexins have also been proposed as therapeutic targets in cancer.[78,96,97] Importantly, this effect was seen to occur via a normalization of the cancer cells not by killing them (cytotoxicity).

Very recently, Mathews *et al.* showed that ion-channel drugs can suppress the cancer phenotype in glioblastoma cell lines.[98] Because glioblastoma overgrowth and drug resistance are characterized by depolarized resting membrane potentials and an acidic extracellular pH in the inter-tumor environment, the authors sought to identify potential treatments by screening a panel of drugs that induce hyperpolarization and/or disrupt proton pumps. They screened varying concentrations of 47 compounds (and combinations thereof), most of which were modulators of ion channels, to identify treatments that suppress cancer phenotypes. The most effective treatments for reducing proliferation, inducing senescence and promoting differentiation in human U87 cells *in vitro* were the following combinations: NS1643 and pantoprazole; retigabine and pantoprazole; and pantoprazole or NS1643 with temozolomide. Because all of these are FDA-approved for other uses, drug repurposing could be a key factor in the development of electroceuticals as an alternative or adjunct to chemotherapy in treatment of glioblastoma and other cancers.

Aging as a morphostasis defect: Bioelectricity as a regulator of cellular and organismal aging

Maintaining order at the tissue level is a highly dynamic task – failures lead to cancer[67,99–101] and disorganization (such as that of the rosettes of the tongue surface when input via the lingual nerve is suppressed[102]). However, the most consistent and pervasive result of the failure of the body's constant regenerative efforts is aging.

Aging in humans is the progressive loss of function of biological processes with a decline of the capacity to maintain anatomical homeostasis and to reproduce, resulting in progressive organ malfunctions, diseases and ultimately death. The traditional conceptual biogerontological framework enunciates that aging is a process of damage accumulation caused by energy metabolism and mitochondrial function, and that cell proliferation and lifespan are limited by replicative senescence caused by telomere shortening.[103] Here, we propose aging as a morphostasis defect; specifically, we hypothesize that it is an ongoing developmental process with probable inputs from the ubiquitous bioelectrical control system that could link the seven pillars of aging[104] (Figure 5a). Next, we discuss some of the potential links between

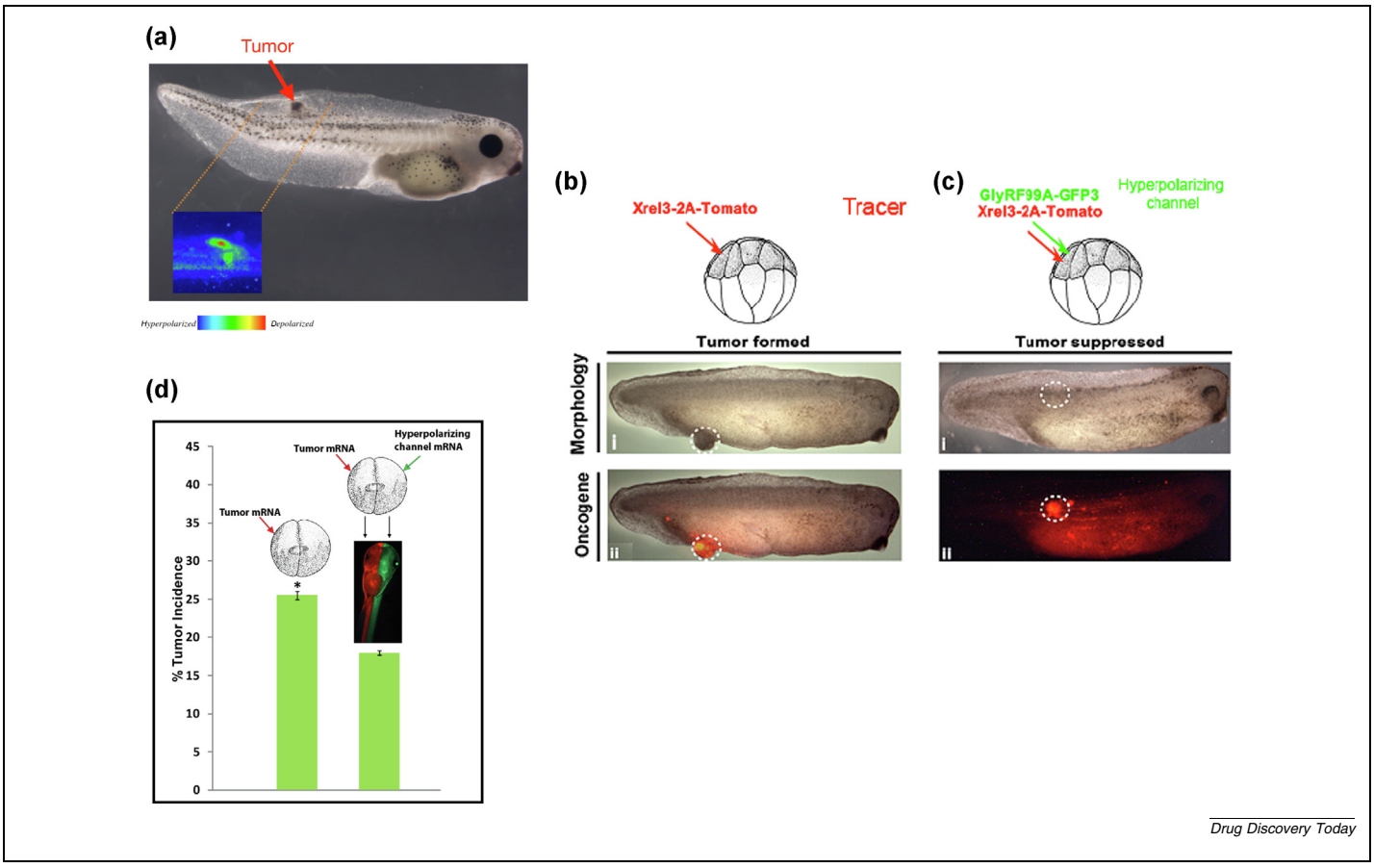

FIGURE 4

Bioelectricity underlies cancer and its treatment. **(a)** Cells during tumorigenesis depolarize at a very early step. The prospective tumor site can be revealed using voltage-sensitive fluorescent dyes. **(b)** Tumorigenesis induced by human oncogenes such as KRAS mutations can be suppressed by manipulating the bioelectric state (b, white dashed circles)[91,92] (bi and bii the location of the tumor with red fluorescent marker on the oncoprotein). **(c)** Tumor formation is suppressed by the co-injection of a hyperpolarizing ion channel (ci). This effect occurs despite the presence of very strong continued expression of the oncoprotein (fluorescently labeled with red signal, cii). **(d)** This suppression can be induced at long distance when the hyperpolarizing channel is injected on the opposite side of the embryo. This shows that the voltage of the environment – the bioelectrical pattern – is fundamental for cell coordination even at a long distance from the tumor.[93,94] Images in a reproduced, with permission, from Refs. 32,91. Panels b and c reproduced, with permission, from Ref. 91.

aging, aging-related diseases and bioelectricity, and the opportunities these create for biomedical research.

The links between aging, aging-related diseases and bioelectricity via ion channels are starting to be discovered.[105–107] Rates of cellular or organismal aging have been shown to be regulated by ion channels. In a few cases, specific types of ion channels and downstream transducers have been associated with aging. The seven pillars of aging can be linked directly or indirectly to bioelectricity.[104] Indeed, ion channels have been associated with metabolism and inflammation during aging.[106] Stem cells and regeneration are controlled by developmental bioelectricity.[17] Epigenetic alterations have been linked to changes in the bioelectrical pattern during tadpole regeneration.[108] Proteostasis is regulated by lysosomal function that is regulated by ion channels.[107] Several stress-regulated ion channels exist[109,110] and macromolecular damage affects ion channels[111] and can impair the bioelectrical pattern responsible for the maintenance of the anatomy. More specifically, ion channels and transporters influence aging and longevity via the regulation of membrane excitability, Ca^{2+} homeostasis, mitochondrial and endolysosomal function, and the transduction of sensory stimuli.[107] Ion channels are also implicated in aging-related diseases impacting the heart, brain, cancer and the eye[105] and in aging-related inflammation via mitochondrial functions.[106] Channelopathies are also often associated with organ failure during aging.[112].

Aging-dependent deterioration of neuronal activity has been linked to changes in Ca^{2+} homeostasis.[113,114] In age-related cardiovascular diseases, Toro *et al.* observed NO release associated with a reduction in the density of the BKCa alpha subunit in coronary smooth muscle,[115] indicating decreased expression during aging. Sinoatrial node (SAN) dysfunction can also be related to changes in the activity of several ion channels that control appropriate directionalities of ionic fluxes in the SAN and cardiac muscle.[105] In addition, studies showed a link between Hutchinson–Gilford progeria syndrome (HGPS) and bioelectricity. An alteration of post-translational modification of progerin that contributes to the phenotype of premature aging in HGPS patients could be caused by an abnormal cytosolic Ca^{2+} concentration owing to upregulation of the transient receptor potential vanilloid type 2 (TRPV2) calcium channel.[116,117]

Rejuvenation strategies including blood factors, metabolic manipulations, senolytics and cellular reprogramming have been

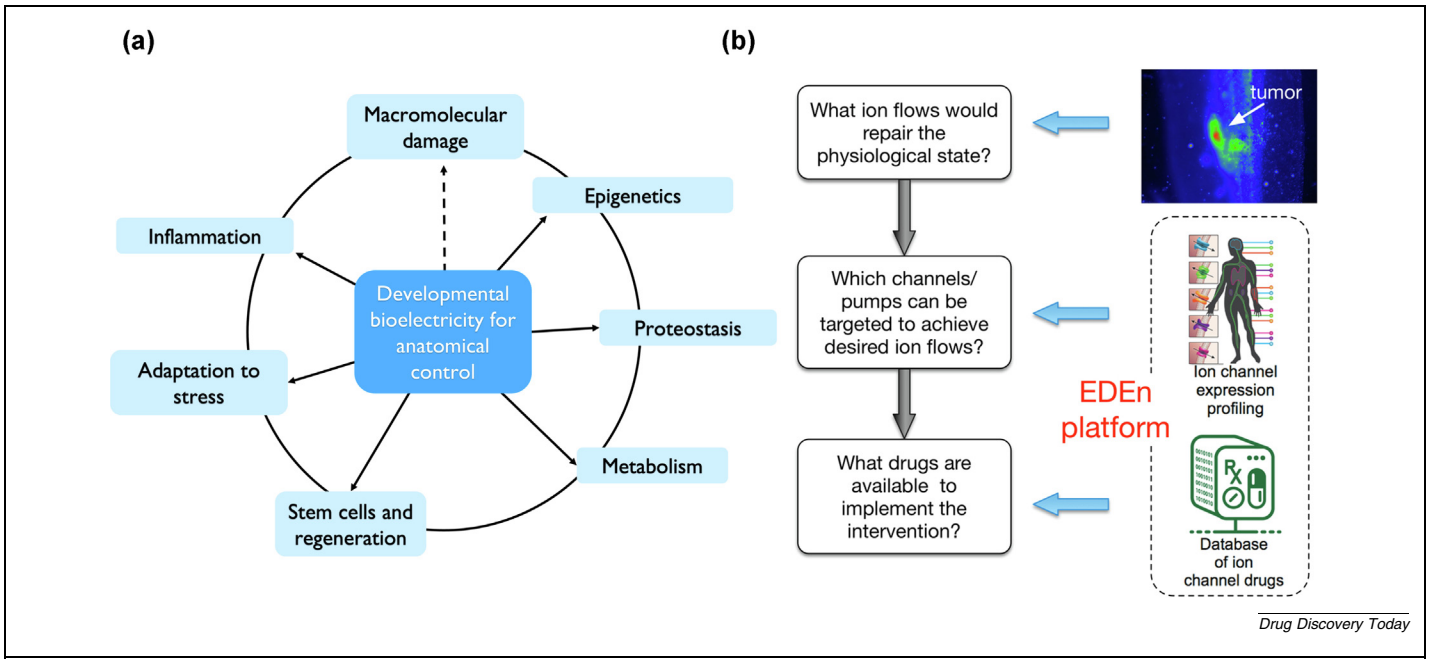

FIGURE 5

Aging as a morphostasis defect: roadmap for morphoceutical discovery. **(a)** In this framework, aging is a developmental defect and as such can be driven by disorders of bioelectricity – a key informational layer for anatomical control. The seven pillars depicted here can be linked to developmental bioelectricity. Solid arrows indicate known mechanistic links; dashed arrows indicate hypothesized links to be tested. **(b)** To change the bioelectrical pattern found in diseases, existing transcriptomic databases can be used to identify potential ion channel targets in any tissue. This result can then be fed as an input to a bioelectrical model to simulate the bioelectrical pattern after intervention and reverse or search the model to determine candidate drugs that will move the current bioelectrical state toward the desired one. This exact strategy was used to identify brain damage repair drugs.[176] Image reproduced, with permission, from Ref. 131.

developed recently to delay and potentially reverse the aging process.[118] Several of these approaches appear to have an impact on bioelectricity and regulate the behavior of ion channels. Metformin acts on nutrient-sensing pathways involved in the rejuvenation effects of dietary regimens[118] and has several effects on bioelectricity. It restores electrophysiology of small conductance calcium-activated potassium channels in the atrium of GK diabetic rats[119] and normalizes the aberrant intracellular Ca^{2+} clearing induced by high glucose in adult rat myocytes.[120] Blood factors can have a pro-rejuvenation effect as shown by the positive impact of young blood on the liver, muscle and brain.[121] Wnt signaling underlying the differentiation bias of aged muscle stem cell is reversed by young blood factors.[122] Wnt signaling influences ion channels and is aberrantly activated in several human diseases including cancer.[123] For example, inhibition of the Wnt signaling pathway downregulates $Ca_V1.2$ in aging models characterized by an altered osteogenic differentiation.[124] More globally, Wnt signaling can be modulated by variation in K^+ and Ca^{2+} fluxes across cell membrane induced by changes in transmembrane potential.[125]

Besides mechanistically testing the links between ion channel disfunction and specific aging processes, it will be essential to determine what happens to the bioelectric prepatterns during aging. What about the bioelectric states that go awry toward the end of the healthspan? Do the bioelectric cues get weaker or are the cues there but the cellular ability to perceive them (and act on them) wanes? In particular, in addition to life extension assays in canonical model systems (mice), it will be essential to understand what happens in apparently immortal species like planaria. It is tempting to hypothesize that their lack of aging, cancer resistance and incredible regenerative capacity all stem from the same thing: remarkably strong tissue-level morphogenetic control mechanisms, which are known to involve bioelectric pattern control.[126].

Concluding remarks and future perspectives: A roadmap for morphoceutical discovery and opportunities

Rational control of bioelectrical states has immense potential for biomedical applications in regeneration, cancer and aging. Several key attributes make bioelectric states an especially attractive target for drug discovery for regeneration, cancer, aging and, indeed, for any disease that can be seen as a defect or dysregulation of morphogenesis.[15,18,27,127] First, many studies demonstrate that bioelectric states constitute a 'master signal', they can control morphogenetic cascades and avoid the need to micromanage extremely complex spatiotemporal biochemical interactions via genetic engineering or classical molecular biology. Instead, accurate generation or regeneration of specific parts can be induced by the simple imposition of specific voltage patterns, as shown for planarian heads,[128] eyes[31] or frog tails,[63] as described above.

Second, bioelectric signaling seems to require only a brief, transient trigger stimulus. For example, in rat mesenchymal cells *in vitro*, a brief electrical stimulus induces long-lasting osteogenic effects, suggesting it could provide powerful synergy with bone-tissue-engineering therapies.[129] Similarly, manipulation of bio-

electrical states for just 1–3 h triggers an 8- or 10-day program of regeneration for planarian heads and tadpole tails, respectively.[35,63]

Third, ion-channel-targeting drugs are the third-best-selling group of prescribed drugs worldwide,[130] providing a large pool of candidates that can be screened for repurposing as electroceuticals. In addition, only a small percentage of the 400 annotated ion-channel genes predicted in the human genome have been targeted, so there are many novel targets for drug discovery as well.

The ideal way to control this native interface is via channel-, pump- and gap-junction-targeting compounds (although light, vibration and other exotic modalities might someday augment this technology). Repurposing of drugs that are already FDA-approved could be particularly expedient for discovery of morphoceuticals. Despite the large number of channel drugs that are already known and tested, this is only a small percentage of ion channels that have been targeted for drug development.[130] Beyond drug repurposing, the *in silico* results on the opening and closing of ion channels will also suggest the design of new compounds using traditional drug design methods, to fill out the massive space of possible electroceuticals.

This effort will require the development of tools that trawl transcriptomic databases (such as EDEn) revealing which ion channels are potential targets for intervention in a specific tissue[131] (Figure 5b). Computational modeling will also be key to simulating bioelectrical dynamics to predict the bioelectrical pattern resulting from opening or closing specific targeted channels with existing drugs. *In silico* results will predict the effects of specific drugs targeting tissue-specific ion channels, suggesting specific *in vivo* experiments to assess their potential for regeneration, repair or cancer normalization.

For a specific purpose, the software will make suggestions regarding which channels to target, and in what way (temporal profile, and which drugs) to obtain the targeted bioelectrical pattern within a tissue. A key ingredient to this workflow however is the development of physiomic datasets – across patients, ages, body regions and health or disease states. When the physiomic data catch up in volume to the deluge of transcriptional, genetic, epigenetic and metabolic datasets that are now available, the next generation of bioelectric interventions will be immediately within reach (especially given the availability of machine learning and data mining tools).

One aspect of this strategy is key. The goal is not to simply find drugs that up- or down-regulate specific channels in the same way one would address over- or under-expressed transcription factors. It is, for example, already known that ion-channel drugs are very promising cancer drugs.[132–138] However, the real promise of this technology will only be realized when they are seen not as rewiring agents mechanically targeting specific gene products or even pathways but as signals (like a keypad depression on a computer interface) designed to manipulate the bioelectrically mediated decision-making of cell networks. This requires a different class of computational models that treats the underlying tissue as a computational agent, not a simple dynamical system.[1,139–141] This in turn suggests the urgency of development (and integration into molecular-biological and physiological models) of computational frameworks, such as causal information theory, that seek to decode the information flow between cells during health and disease[75,141–143] or integrative network representation for drug discovery[144,145] including the transcriptional representation of the electrome using EDEn for example.[131]

A second aspect is the non-locality of bioelectric information.[146] As in the brain, several disease states receive input from distant locations. For example, embryonic brain structure can be damaged and repaired by bioelectric states occurring on the other side of the animal.[147,148] The same is true for tumorigenesis[149,150] and the amputation status of one limb can be read out from the bioelectric signals of the contralateral (untouched) limb.[151] This suggests the possibility of surrogate site diagnostics and treatment, and makes it imperative that bioelectric models and drug searches do not focus on the local site of a wound or defect but consider the entire organism as a potential interface for treatment. For example, in the case of tail regeneration[63] and hyperinnervation to improve vision,[152] the entire animal was exposed but only relevant cells responded (i.e., no need for spatially specific stimulation). In other cases, local delivery via a wearable bioreactor was used.[64,65]

More broadly, beyond electroceuticals, what binds biochemical, biomechanical, optical and bioelectrical morphoceuticals together is the commitment to exploiting high-level problem-solving capacities in cells and tissues. For example, other physiological parameters such as REDOX state[153,154] or tissue biomechanics[155,156] can likewise serve as master controls for complex outcomes. But whether induced by CRISPR, optogenetic stimulation, pharmacological agents or even behavioral cues,[157,158] the crucial aspect is to move from managing micro-level components to the cybernetic properties of entire circuits.[159–161]

One implication of top-down control of modules and the allostatic capabilities of living matter[1,162] is that dosage and timing might not have to be micromanaged. For example, in recent examples of triggering the regeneration of complex organs and appendages via bioelectric,[35] hormonal[65] and other biochemical means,[64] the organ type, composition, shape and size were all induced without any attempts to identify an effective dose or precise timing regime. A key part of this strategy is offloading the complexity onto the organism itself (exploiting its modular, regulative control structure) to achieve complex outcomes where precise parameters and molecular details are not yet known. Morphoceuticals can be rationally designed based on top-down control mechanisms[4,19] or discovered in pharmacological[30,163,164] or genetic[44,46] screens specifically focused on high-level control phenotypes.

Taken together, a picture emerges in which the regenerative medicine of the future can stand on the shoulders of two giants: computer science and cognitive/behavioral science. Computational neuroscience progress reveals how a multiscale approach to the brain (not only molecular but circuit, network and even psychology-level) is providing a full-stack understanding of the control of behavior. We suggest that the same strategy can be applied in regenerative medicine, by targeting bioelectricity as the computational glue that binds cells together to common purpose *in vivo*, and embracing the idea that causally effective inter-

ventions can be applied at levels higher than that of molecules.[6,141,165,166] Definitive approaches to aging, cancer, traumatic injury and birth defects await, limited only by our ability to see tissues as competent information-processing agents that we now have the tools to meet at their own level.

Data availability

No data was used for the research described in the article.

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Conflicts of interest

M.L. is a co-founder of Morphoceuticals Inc., a company seeking to develop bioelectric approaches to regeneration.

References

1. Levin M. Collective Intelligence of Morphogenesis as a Teleonomic Process. In: Corning PA, Kauffman SA, Noble D, Shapiro JA, Vane-Wright RI, Pross A, eds. *Evolution "on Purpose": Teleonomy in Living Systems*. MIT Press; 2023:175–198.
2. Vandenberg LN et al.. Normalized shape and location of perturbed craniofacial structures in the Xenopus tadpole reveal an innate ability to achieve correct morphology. *Dev Dyn*. 2012;241:863–878.
3. Fields C, Levin M. Competency in Navigating Arbitrary Spaces as an Invariant for Analyzing Cognition in Diverse Embodiments. *Entropy (Basel)*. 2022;24.
4. Pezzulo G, Levin M. Top-down models in biology: explanation and control of complex living systems above the molecular level. *J R Soc Interface*. 2016;13.
5. Harris AK. The need for a concept of shape homeostasis. *Biosystems*. 2018;173:65–72.
6. Mathews J, Levin M. The body electric 2.0: recent advances in developmental bioelectricity for regenerative and synthetic bioengineering. *Curr Opin Biotechnol*. 2018;52:134–144.
7. Jia MP et al.. Bioelectronic control of chloride ions and concentration with Ag/AgCl contacts. *APL Mater*. 2020;8.
8. Ovens L. Electrical stimulation therapy and electroceutical treatment for the management of venous leg ulcers. *Br J Community Nurs*. 2017;22:S28–S36.
9. Tuszynski J et al.. Ion Channel and Neurotransmitter Modulators as Electroceutical Approaches to the Control of Cancer. *Curr Pharm Des*. 2017;23:4827–4841.
10. Sen CK et al.. Electroceutical Management of Bacterial Biofilms and Surgical Infection. *Antioxid Redox Signal*. 2020;33:713–724.
11. Famm K et al.. Drug discovery: a jump-start for electroceuticals. *Nature*. 2013;496:159–161.
12. Pavlov VA, Tracey KJ. Bioelectronic medicine: Preclinical insights and clinical advances. *Neuron*. 2022;110:3627–3644.
13. Bhavsar MB et al.. Role of Bioelectricity During Cell Proliferation in Different Cell Types. *Front Bioeng Biotechnol*. 2020;8:603.
14. Oliveira KMC et al.. Electrical stimulation shifts healing/scarring towards regeneration in a rat limb amputation model. *Sci Rep*. 2019;9:11433.
15. Leppik L et al.. Combining electrical stimulation and tissue engineering to treat large bone defects in a rat model. *Sci Rep*. 2018;8:6307.
16. Harris MP. Bioelectric signaling as a unique regulator of development and regeneration. *Development*. 2021;148.
17. Levin M. Bioelectric signaling: Reprogrammable circuits underlying embryogenesis, regeneration, and cancer. *Cell*. 2021;184:1971–1989.
18. Bates E. Ion Channels in Development and Cancer. *Annu Rev Cell Dev Biol*. 2015;31:231–247.
19. Pezzulo G, Levin M. Re-membering the body: applications of computational neuroscience to the top-down control of regeneration of limbs and other complex organs. *Integr Biol (Camb)*. 2015;7:1487–1517.
20. Fields C et al.. Morphological Coordination: A Common Ancestral Function Unifying Neural and Non-Neural Signaling. *Physiology*. 2020;35:16–30.
21. Levin M. The Computational Boundary of a "Self": Developmental Bioelectricity Drives Multicellularity and Scale-Free Cognition. *Front Psychol*. 2019;10:2688.
22. Sundelacruz S et al.. Membrane Potential Depolarization Alters Calcium Flux and Phosphate Signaling During Osteogenic Differentiation of Human Mesenchymal Stem Cells. *Bioelectricity*. 2019;1:56–66.
23. Sundelacruz S et al.. Comparison of the depolarization response of human mesenchymal stem cells from different donors. *Sci Rep*. 2015;5:18279.
24. Sundelacruz S et al.. Depolarization alters phenotype, maintains plasticity of predifferentiated mesenchymal stem cells. *Tissue Eng A*. 2013;19:1889–1908.
25. Sundelacruz S et al.. Membrane potential controls adipogenic and osteogenic differentiation of mesenchymal stem cells. *PLoS One*. 2008;3:e3737.
26. Levin M. Molecular bioelectricity: how endogenous voltage potentials control cell behavior and instruct pattern regulation in vivo. *Mol Biol Cell*. 2014;25:3835–3850.
27. Levin M, Martyniuk CJ. The bioelectric code: An ancient computational medium for dynamic control of growth and form. *Biosystems*. 2018;164:76–93.
28. Sundelacruz S et al.. Role of membrane potential in the regulation of cell proliferation and differentiation. *Stem Cell Rev Rep*. 2009;5:231–246.
29. McLaughlin KA, Levin M. Bioelectric signaling in regeneration: Mechanisms of ionic controls of growth and form. *Dev Biol*. 2018;433:177–189.
30. Sullivan KG, Levin M. Neurotransmitter signaling pathways required for normal development in Xenopus laevis embryos: a pharmacological survey screen. *J Anat*. 2016;229:483–502.
31. Pai VP et al.. Transmembrane voltage potential controls embryonic eye patterning in Xenopus laevis. *Development*. 2012;139:313–323.
32. Vandenberg LN et al.. V-ATPase-dependent ectodermal voltage and pH regionalization are required for craniofacial morphogenesis. *Dev Dyn*. 2011;240:1889–1904.
33. Adams DS et al.. Bioelectric signalling via potassium channels: a mechanism for craniofacial dysmorphogenesis in KCNJ2-associated Andersen-Tawil Syndrome. *J Physiol*. 2016;594:3245–3270.
34. Pezzulo G et al.. (2021) Bistability of somatic pattern memories: stochastic outcomes in bioelectric circuits underlying regeneration. *Philos Trans R Soc Lond B Biol Sci*. 1821;376:20190765.
35. Durant F et al.. The Role of Early Bioelectric Signals in the Regeneration of Planarian Anterior/Posterior Polarity. *Biophys J*. 2019;116:948–961.
36. Durant F et al.. Physiological controls of large-scale patterning in planarian regeneration: a molecular and computational perspective on growth and form. *Regeneration (Oxf)*. 2016;3:78–102.
37. Emmons-Bell M et al.. Gap Junctional Blockade Stochastically Induces Different Species-Specific Head Anatomies in Genetically Wild-Type Girardia dorotocephala Flatworms. *Int J Mol Sci*. 2015;16:27865–27896.
38. Srivastava P, Kane A, Harrison C, Levin M. A Meta-Analysis of Bioelectric Data in Cancer, Embryogenesis, and Regeneration. *Bioelectricity*.. 2021;3:42–67. https://doi.org/10.1089/bioe.2019.0034. Epub 2021 Mar 16. PMID: 34476377; PMCID: PMC8370481.
39. Sun AX et al.. Potassium channel dysfunction in human neuronal models of Angelman syndrome. *Science*. 2019;366:1486–1492.
40. Smith RS et al.. Sodium Channel SCN3A (NaV1.3) Regulation of Human Cerebral Cortical Folding and Oral Motor Development. *Neuron*. 2018;99:905–913 e907.
41. Bauer CK et al.. Mutations in KCNK4 that Affect Gating Cause a Recognizable Neurodevelopmental Syndrome. *Am J Hum Genet*. 2018;103:621–630.
42. Masotti A et al.. Keppen-Lubinsky Syndrome Is Caused by Mutations in the Inwardly Rectifying K(+) Channel Encoded by KCNJ6. *Am J Hum Genet*. 2015;96:295–300.
43. Kortum F et al.. Mutations in KCNH1 and ATP6V1B2 cause Zimmermann-Laband syndrome. *Nat Genet*. 2015;47:661–667.
44. George LF et al.. Ion Channel Contributions to Wing Development in Drosophila melanogaster. *G3 (Bethesda)*. 2019;9:999–1008.
45. Yi C et al.. A calcineurin-mediated scaling mechanism that controls a K(+)-leak channel to regulate morphogen and growth factor transcription. *Elife*. 2021;10.
46. Perathoner S et al.. Bioelectric signaling regulates size in zebrafish fins. *PLoS Genet*. 2014;10:e1004080.
47. Levin M. Bioelectrical approaches to cancer as a problem of the scaling of the cellular self. *Prog Biophys Mol Biol*. 2021;165:102–113.
48. Feng JF et al.. Electrical Guidance of Human Stem Cells in the Rat Brain. *Stem Cell Rep*. 2017;9:177–189.

49. Zajdel TJ et al.. SCHEEPDOG: Programming Electric Cues to Dynamically Herd Large-Scale Cell Migration. *Cell Syst*. 2020;10:506–514 e503.
50. Song B et al.. Electric signals counterbalanced posterior vs anterior PTEN signaling in directed migration of Dictyostelium. *Cell Biosci*. 2021;11:111.
51. Mehta AS et al.. Physiological electric fields induce directional migration of mammalian cranial neural crest cells. *Dev Biol*. 2021;471:97–105.
52. Jiang T-X et al.. Global feather orientations changed by electric current. *iScience*. 2021;24 102671.
53. Zhao S et al.. Biomedical applications of electrical stimulation. *Cell Mol Life Sci*. 2020;77:2681–2699.
54. Pai VP et al.. HCN2 Rescues brain defects by enforcing endogenous voltage pre-patterns. *Nat Commun*. 2018;9:998.
55. Pai VP et al.. Endogenous gradients of resting potential instructively pattern embryonic neural tissue via Notch signaling and regulation of proliferation. *J Neurosci*. 2015;35:4366–4385.
56. Pai VP et al.. HCN2 Channel-Induced Rescue of Brain Teratogenesis via Local and Long-Range Bioelectric Repair. *Front Cell Neurosci*. 2020;14:136.
57. Pai VP, Levin M. HCN2 channel-induced rescue of brain, eye, heart and gut teratogenesis caused by nicotine, ethanol and aberrant notch signalling. *Wound Repair Regen*. 2022.
58. Pai VP et al.. HCN2 Channel-Induced Rescue of Brain Teratogenesis via Local and Long-Range Bioelectric Repair. *Front Cell Neurosci*. 2020;14:136.
59. Leppik LP et al.. Effects of electrical stimulation on rat limb regeneration, a new look at an old model. *Sci Rep*. 2015;5:1–9.
60. Sisken BF et al.. Response of amputated rat limbs to fetal nerve tissue implants and direct current. *J Orthop Res*. 1984;2:177–189.
61. Becker RO, Spadaro JA. Electrical stimulation of partial limb regeneration in mammals. *Bull N Y Acad Med*. 1972;48:627–641.
62. Libbin RM et al.. Partial regeneration of the above-elbow amputated rat forelimb. II. Electrical and mechanical facilitation. *J Morphol*. 1979;159:439–452.
63. Tseng AS et al.. Induction of vertebrate regeneration by a transient sodium current. *J Neurosci*. 2010;30:13192–13200.
64. Murugan NJ et al.. Acute multidrug delivery via a wearable bioreactor facilitates long-term limb regeneration and functional recovery in adult Xenopus laevis. *Sci Adv*. 2022;8:eabj2164.
65. Herrera-Rincon C et al.. Brief Local Application of Progesterone via a Wearable Bioreactor Induces Long-Term Regenerative Response in Adult Xenopus Hindlimb. *Cell Rep*. 2018;25:1593-+.
66. Levin M. Bioelectrical approaches to cancer as a problem of the scaling of the cellular self. *Prog Biophys Mol Biol*. 2021;165:102–113.
67. Rubin H. Cancer as a dynamic developmental disorder. *Cancer Res*. 1985;45:2935–2942.
68. Bussey KJ et al.. Ancestral gene regulatory networks drive cancer. *PNAS*. 2017;114:6160–6162.
69. Davies PCW, Lineweaver CH. Cancer tumors as Metazoa 1.0: tapping genes of ancient ancestors. *Phys Biol*. 2011;8 015001.
70. Levin M. The Computational Boundary of a "Self": Developmental Bioelectricity Drives Multicellularity and Scale-Free Cognition. *Front Psychol*. 2019;10:2688.
71. Aasen T et al.. The relationship between connexins, gap junctions, tissue architecture and tumour invasion, as studied in a novel in vitro model of HPV-16-associated cervical cancer progression. *Oncogene*. 2003;22:7969–7980.
72. Chernet BT, Levin M. Endogenous Voltage Potentials and the Microenvironment: Bioelectric Signals that Reveal, Induce and Normalize Cancer. *J Clin Exp Oncology*. 2013.
73. Loch-Caruso R, Trosko JE. Inhibited intercellular communication as a mechanistic link between teratogenesis and carcinogenesis. *Crit Rev Toxicol*. 1985;16:157–183.
74. Trosko JE et al.. Inhibition of cell-cell communication by tumor promoters. *Carcinog Compr Surv*. 1982;7:565–585.
75. Quicke P et al.. Voltage imaging reveals the dynamic electrical signatures of human breast cancer cells. *Commun Biol*. 2022;5:1178.
76. Leslie TK et al.. Sodium homeostasis in the tumour microenvironment. *Biochim Biophys Acta Rev Cancer*. 2019;1872 188304.
77. Jansson B. Geographic cancer risk and intracellular potassium/sodium ratios. *Cancer Detect Prev*. 1986;9:171–194.
78. Kandouz M, Batist G. Gap junctions and connexins as therapeutic targets in cancer. *Expert Opin Ther Targets*. 2010;14:681–692.
79. Moore D et al.. Cancer as a disorder of patterning information: computational and biophysical perspectives on the cancer problem. *Convergent Sci Phys Oncol*. 2017;3 043001.
80. Blackiston D et al.. Transmembrane potential of GlyCl-expressing instructor cells induces a neoplastic-like conversion of melanocytes via a serotonergic pathway. *Dis Model Mech*. 2011;4:67–85.
81. Lobikin M et al.. Serotonergic regulation of melanocyte conversion: A bioelectrically regulated network for stochastic all-or-none hyperpigmentation. *Sci Signal*. 2015;8:ra99.
82. Prevarskaya N et al.. Ion Channels in Cancer: Are Cancer Hallmarks Oncochannelopathies?. *Physiol Rev*. 2018;98:559–621.
83. Yang M, Brackenbury WJ. Membrane potential and cancer progression. *Front Physiol*. 2013;4:185.
84. Yang M et al.. Therapeutic potential for phenytoin: targeting Na(v)1.5 sodium channels to reduce migration and invasion in metastatic breast cancer. *Breast Cancer Res Treatm*. 2012;134:603–615.
85. Brackenbury WJ. Voltage-gated sodium channels and metastatic disease. *Channels*. 2012;6:352–361.
86. Hutchings C et al.. (2020) Nerve input to tumours: Pathophysiological consequences of a dynamic relationship. *Biochim Biophys Acta Rev Cancer*. 1874;2 188411.
87. Djamgoz MB et al.. Ion transport and cancer: from initiation to metastasis. *Philos Trans R Soc Lond Ser B, Biol Sci*. 2014;369 20130092.
88. Payne SL et al.. Bioelectric Control of Metastasis in Solid Tumors. *Bioelectricity*. 2019;1:114–130.
89. Oudin MJ, Weaver VM. Physical and Chemical Gradients in the Tumor Microenvironment Regulate Tumor Cell Invasion, Migration, and Metastasis. *Cold Spring Harb Symp Quant Biol*. 2016;81:189–205.
90. Mesnil M et al.. Role of blocked gap junctional intercellular communication in non-genotoxic carcinogenesis. *Toxicol Lett*. 1995;82–83:701–706.
91. Chernet BT, Levin M. Transmembrane voltage potential is an essential cellular parameter for the detection and control of tumor development in a Xenopus model. *Dis Model Mech*. 2013;6:595–607.
92. Chernet BT et al.. Use of genetically encoded, light-gated ion translocators to control tumorigenesis. *Oncotarget*. 2016;7:19575–19588.
93. Chernet BT et al.. Long-range gap junctional signaling controls oncogene-mediated tumorigenesis in Xenopus laevis embryos. *Front Physiol*. 2015;5:519.
94. Chernet BT, Levin M. Transmembrane voltage potential of somatic cells controls oncogene-mediated tumorigenesis at long-range. *Oncotarget*. 2014;5:3287–3306.
95. Altamura C et al.. Ion Channel Involvement in Tumor Drug Resistance. *J Pers Med*. 2022;12.
96. Defamie N et al.. Connexins, gap junctions and tissue invasion. *FEBS Lett*. 2014;588:1331–1338.
97. Yamasaki H et al.. Intercellular communication and carcinogenesis. *Mutat Res/Fundam Mol Mech Mutagen*. 1995;333:181–188.
98. Mathews J et al.. Ion Channel Drugs Suppress Cancer Phenotype in NG108-15 and U87 Cells: Toward Novel Electroceuticals for Glioblastoma. *Cancers (Basel)*. 2022;14:1499.
99. Rubin H et al.. Cellular aging, destabilization, and cancer. *Proc Natl Acad Sci U S A*. 1996;93:1825–1830.
100. Rubin H. What keeps cells in tissues behaving normally in the face of myriad mutations?. *Bioessays*. 2006;28:515–524.
101. Rubin H. Ordered heterogeneity and its decline in cancer and aging. *Adv Cancer Res*. 2007;98:117–147.
102. Nagato T et al.. Effect of denervation on morphogenesis of the rat fungiform papilla. *Acta Anat (Basel)*. 1995;153:301–309.
103. Gems D, de Magalhaes JP. The hoverfly and the wasp: A critique of the hallmarks of aging as a paradigm. *Ageing Res Rev*. 2021;70 101407.
104. Kennedy BK et al.. Geroscience: linking aging to chronic disease. *Cell*. 2014;159:709–713.
105. Rao V et al.. Ion channels in aging and aging-related diseases. *Mol Mech Aging Process Rejuvenation*. 2016.
106. Strickland M et al.. Relationships Between Ion Channels, Mitochondrial Functions and Inflammation in Human Aging. *Front Physiol*. 2019;10:158.
107. Venkatachalam K. Regulation of Aging and Longevity by Ion Channels and Transporters. *Cells*. 2022;11:1180.
108. Tseng AS, Levin M. Transducing bioelectric signals into epigenetic pathways during tadpole tail regeneration. *Anat Rec (Hoboken)*. 2012;295:1541–1551.
109. Knowles H et al.. The TRPM2 ion channel, an oxidative stress and metabolic sensor regulating innate immunity and inflammation. *Immunol Res*. 2013;55:241–248.
110. Ramírez A et al.. Ion Channels and Oxidative Stress as a Potential Link for the Diagnosis or Treatment of Liver Diseases. *Oxid Med Cell Longev*. 2016;2016:3928714.

111. Heijman J, Dobrev D. Ion channels as part of macromolecular multiprotein complexes : Clinical significance. *Herzschrittmacherther Elektrophysiol*. 2018;29:30–35.
112. Kass RS. The channelopathies: novel insights into molecular and genetic mechanisms of human disease. *J Clin Invest*. 2005;115:1986–1989.
113. Thibault O et al.. Expansion of the calcium hypothesis of brain aging and Alzheimer's disease: minding the store. *Aging Cell*. 2007;6:307–317.
114. Wojda U et al.. Calcium ions in neuronal degeneration. *IUBMB Life*. 2008;60:575–590.
115. Toro L et al.. Aging, ion channel expression, and vascular function. *Vascul Pharmacol*. 2002;38:73–80.
116. Kalinowski A et al.. Calcium causes a conformational change in lamin a tail domain that promotes farnesylmediated membrane association. *Biophys J*. 2013;104:2246–2253.
117. Lo CY et al.. An upregulation in the expression of vanilloid transient potential channels 2 enhances hypotonicity-induced cytosolic Ca(2)(+) rise in human induced pluripotent stem cell model of Hutchinson-Gillford Progeria. *PLoS One*. 2014;9:e87273.
118. Mahmoudi S et al.. Turning back time with emerging rejuvenation strategies. *Nat Cell Biol*. 2019;21:32–43.
119. Fu X et al.. Metformin restores electrophysiology of small conductance calcium-activated potassium channels in the atrium of GK diabetic rats. *BMC Cardiovasc Disord*. 2018;18:63.
120. Ren J et al.. Metformin but not glyburide prevents high glucose-induced abnormalities in relaxation and intracellular Ca2+ transients in adult rat ventricular myocytes. *Diabetes*. 1999;48:2059–2065.
121. Rebo J et al.. A single heterochronic blood exchange reveals rapid inhibition of multiple tissues by old blood. *Nat Commun*. 2016;7:13363.
122. Brack AS et al.. A temporal switch from Notch to Wnt signaling in muscle stem cells is necessary for normal adult myogenesis. *Cell Stem Cell*. 2008;2:50–59.
123. Muccioli S et al.. From Channels to Canonical Wnt Signaling: A Pathological Perspective. *Int J Mol Sci*. 2021;22:4613.
124. Fei D et al.. Cav1. 2 regulates osteogenesis of bone marrow-derived mesenchymal stem cells via canonical wnt pathway in age-related osteoporosis. *Aging Cell*. 2019;18:e12967.
125. Breuer EK et al.. Potassium channel activity controls breast cancer metastasis by affecting beta-catenin signaling. *Cell Death Dis*. 2019;10:180.
126. Levin M et al.. Planarian regeneration as a model of anatomical homeostasis: Recent progress in biophysical and computational approaches. *Semin Cell Dev Biol*. 2018;87:125–144.
127. McCaig CD et al.. Controlling cell behavior electrically: current views and future potential. *Physiol Rev*. 2005;85:943–978.
128. Beane WS et al.. A Chemical Genetics Approach Reveals H, K-ATPase-Mediated Membrane Voltage Is Required for Planarian Head Regeneration. *Chem Biol*. 2011;18:77–89.
129. Eischen-Loges M et al.. Pretreating mesenchymal stem cells with electrical stimulation causes sustained long-lasting pro-osteogenic effects. *PeerJ*. 2018;6: e4959.
130. Wilkinson TC et al.. Discovery of functional antibodies targeting ion channels. *J Biomol Screen*. 2015;20:454–467.
131. Churchill CDM et al.. EDEn-Electroceutical Design Environment: Ion Channel Tissue Expression Database with Small Molecule Modulators. *iScience*. 2019;11:42–56.
132. Koltai T. Voltage-gated sodium channel as a target for metastatic risk reduction with re-purposed drugs. *F1000Res*. 2015;4:297.
133. Kale VP et al.. Targeting ion channels for cancer therapy by repurposing the approved drugs. *Biochim Biophys Acta*. 2015;1848:2747–2755.
134. Fairhurst C et al.. Sodium channel-inhibiting drugs and survival of breast, colon and prostate cancer: a population-based study. *Sci Rep*. 2015;5:16758.
135. Arcangeli A, Becchetti A. New Trends in Cancer Therapy: Targeting Ion Channels and Transporters. *Pharmaceuticals*. 2010;3:1202.
136. Arcangeli A et al.. Targeting ion channels in cancer: a novel frontier in antineoplastic therapy. *Curr Med Chem*. 2009;16:66–93.
137. Arcangeli A et al.. Targeting ion channels in leukemias: a new challenge for treatment. *Curr Med Chem*. 2012;19:683–696.
138. Arcangeli A. Ion channels and transporters in cancer. 3. Ion channels in the tumor cell-microenvironment cross talk. *Am J Physiol Cell Physiol*. 2011;301: C762–C771.
139. Pietak A, Levin M. Bioelectric gene and reaction networks: computational modelling of genetic, biochemical and bioelectrical dynamics in pattern regulation. *J R Soc Interface*. 2017;14.
140. Riol A et al.. Cell Systems Bioelectricity: How Different Intercellular Gap Junctions Could Regionalize a Multicellular Aggregate. *Cancers (Basel)*. 2021;13.
141. Moore D et al.. Cancer as a disorder of patterning information: computational and biophysical perspectives on the cancer problem. *Convergent Sci Phys Oncol*. 2017;3 043001.
142. McMillen P et al.. Toward Decoding Bioelectric Events in Xenopus Embryogenesis: New Methodology for Tracking Interplay Between Calcium and Resting Potentials In Vivo. *J Mol Biol*. 2020;432:605–620.
143. McMillen P et al.. Information Theory as an Experimental Tool for Integrating Disparate Biophysical Signaling Modules. *Int J Mol Sci*. 2022;23.
144. Pio-Lopez L et al.. MultiVERSE: a multiplex and multiplex-heterogeneous network embedding approach. *Sci Rep*. 2021;11:8794.
145. Halu A et al.. The multiplex network of human diseases. *NPJ Syst Biol Appl*. 2019;5:15.
146. McMillen P et al.. Beyond Neurons: Long Distance Communication in Development and Cancer. *Front Cell Dev Biol*. 2021;9 739024.
147. Pai VP et al.. Endogenous gradients of resting potential instructively pattern embryonic neural tissue via Notch signaling and regulation of proliferation. *J Neurosci*. 2015;35:4366–4385.
148. Pai VP et al.. Local and long-range endogenous resting potential gradients antagonistically regulate apoptosis and proliferation in the embryonic CNS. *Int J Dev Biol*. 2015;59:327–340.
149. Chernet BT et al.. Long-range gap junctional signaling controls oncogene-mediated tumorigenesis in Xenopus laevis embryos. *Front Physiol*. 2015;5:519.
150. Chernet BT, Levin M. Transmembrane voltage potential of somatic cells controls oncogene-mediated tumorigenesis at long-range. *Oncotarget*. 2014;5:3287–3306.
151. Busse SM et al.. Cross-limb communication during Xenopus hindlimb regenerative response: non-local bioelectric injury signals. *Development*. 2018;145.
152. Blackiston DJ et al.. A novel method for inducing nerve growth via modulation of host resting potential: gap junction-mediated and serotonergic signaling mechanisms. *Neurotherapeutics*. 2015;12:170–184.
153. Ferreira F et al.. Early redox activities modulate Xenopus tail regeneration. *Nat Commun*. 2018;9:4296.
154. Ferreira F et al.. Early bioelectric activities mediate redox-modulated regeneration. *Development*. 2016;143:4582–4594.
155. Mammoto T et al.. Mechanochemical control of mesenchymal condensation and embryonic tooth organ formation. *Dev Cell*. 2011;21:758–769.
156. Mammoto T, Ingber DE. Mechanical control of tissue and organ development. *Development*. 2010;137:1407–1420.
157. Lui F et al.. Neural bases of conditioned placebo analgesia. *Pain*. 2010;151:816–824.
158. Colloca L, Benedetti F. Placebos and painkillers: is mind as real as matter?. *Nat Rev Neurosci*. 2005;6:545–552.
159. Csermely P et al.. Learning of Signaling Networks: Molecular Mechanisms. *Trends Biochem Sci*. 2020;45:284–294.
160. Perez-Lopez AR et al.. Targets of drugs are generally, and targets of drugs having side effects are specifically good spreaders of human interactome perturbations. *Sci Rep*. 2015;5:10182.
161. Mathews, J. et al. (2022). Cellular Signaling Pathways as Plastic, Proto-cognitive Systems: Implications for Biomedicine.
162. Davies J, Levin M. Synthetic morphology via active and agential matter. *Nature Bioeng*. 2022.
163. Sullivan KG, Levin M. Inverse Drug Screening of Bioelectric Signaling and Neurotransmitter Roles: Illustrated Using a Xenopus Tail Regeneration Assay. *Cold Spring Harb Protoc*. 2018;2018. pdb prot099937.
164. Adams DS, Levin M. Inverse drug screens: a rapid and inexpensive method for implicating molecular targets. *Genesis*. 2006;44:530–540.
165. Hoel EP et al.. Quantifying causal emergence shows that macro can beat micro. *Proc Natl Acad Sci USA*. 2013;110:19790–19795.
166. Moore DG et al.. Inform: Efficient Information-Theoretic Analysis of Collective Behaviors. *Front Robot AI*. 2018;5:60.
167. Levin M et al.. Asymmetries in H+/K+-ATPase and cell membrane potentials comprise a very early step in left-right patterning. *Cell*. 2002;111:77–89.
168. Cairns DM et al.. Ivermectin Promotes Peripheral Nerve Regeneration during Wound Healing. *ACS Omega*. 2018;3:12392–12402.
169. Ferenc NN, Levin M. Effects of Ivermectin Exposure on Regeneration of D. dorotocephala Planaria: Exploiting Human-Approved Ion Channel Drugs as Morphoceuticals. *Macromol Biosci*. 2019;19:e1800237.

170. Dolk H et al.. Lamotrigine use in pregnancy and risk of orofacial cleft and other congenital anomalies. *Neurology*. 2016;86:1716–1725.
171. Lin G et al.. Imparting regenerative capacity to limbs by progenitor cell transplantation. *Dev Cell*. 2013;24:41–51.
172. Fukumoto T et al.. Serotonin transporter function is an early step in left-right patterning in chick and frog embryos. *Dev Neurosci*. 2005;27:349–363.
173. Frey E et al.. TRPV1 Agonist, Capsaicin, Induces Axon Outgrowth after Injury via Ca(2+)/PKA Signaling. *eNeuro*. 2018;5.
174. Cervera J et al.. From non-excitable single-cell to multicellular bioelectrical states supported by ion channels and gap junction proteins: Electrical potentials as distributed controllers. *Prog Biophys Mol Biol*. 2019;149:39–53.
175. Cervera J et al.. Bioelectrical coupling in multicellular domains regulated by gap junctions: A conceptual approach. *Bioelectrochemistry*. 2018;123:45–61.
176. Pai VP et al.. HCN2 Rescues brain defects by enforcing endogenous voltage pre-patterns. *Nat Commun*. 2018;9:998.

POST-SCREEN (GREY)