Re-Membering the Body: applications of computational neuroscience to the top-down control of regeneration of limbs and other complex organs

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Some animals regenerate limbs and remodel complex organs. Despite progress in molecular biology, we still lack understanding of the remarkable coordination of cell activity towards a large-scale anatomical outcome, stopping when target morphology is achieved. Cognitive neuroscience offers a paradigm for how cellular networks store memories of specific shapes and pursue goal states. We propose that these key insights map closely onto regenerative biology. Advances in developmental bioelectricity reveal that all cells could form networks using electrical communication to store and implement shape memories. We propose that bioelectricity is a nexus that shows how shape homeostasis can be implemented in somatic networks, and suggests tractable new approaches for increased control of growth and form in regenerative medicine and synthetic bioengineering.

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Re-Membering the Body: applications of computational neuroscience to the top-down control of regeneration of limbs and other complex organs

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A major goal of regenerative medicine and bioengineering is the regeneration of complex organs, such as limbs, and the capability to create artificial constructs (so-called biobots) with defined morphologies and robust self-repair capabilities. Developmental biology presents remarkable examples of systems that self-assemble and regenerate complex structures toward their correct shape despite significant perturbations. A fundamental challenge is to translate progress in molecular genetics into control of large-scale organismal anatomy, and the field is still searching for an appropriate theoretical paradigm for facilitating control of pattern homeostasis. However, computational neuroscience provides many examples in which cell networks (brains) store memories of geometrical states and coordinate their activity towards proximal and distant goals. In this Perspective, we propose that programming large-scale morphogenesis requires exploiting the information processing by which cellular structures work toward specific shapes. In non-neural cells, as in the brain, bioelectric signaling implements information processing, decision-making, and memory in regulating pattern and its remodeling. Thus, approaches used in computational neuroscience to understand goal-seeking neural systems offer a toolbox of techniques to model and control regenerative pattern formation. Here, we review recent data on developmental bioelectricity as a regulator of patterning, and propose that target morphology could be encoded within tissues as a kind of memory, using the same molecular mechanisms and algorithms so successfully exploited by the brain. We highlight the next steps of an unconventional research program, which may allow top-down control of growth and form for numerous applications in regenerative medicine and synthetic bioengineering.

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1. Introduction

1.1. *The challenge of next-generation regenerative bioengineering*

A key goal in regenerative medicine is to replace damaged or aging organs, for example the repair of entire amputated limbs [1]. Taking the control of biological growth and form to its ultimate conclusion, bioengineering hopes to eventually be able to make self-repairing living structures in any desired configuration – the so-called "biobots" (bioengineered hybrid constructs with specific morphology and function) [2]. However, even when it becomes possible to make any cell type from stem cells, how would we restore a complete hand or eye? Micromanaging the construction process at the lowest level is likely not feasible for such complex structures. A teratoma tumor may possess hair, teeth, and muscle, but lacks appropriate 3D organization, demonstrating that well-differentiated cell types are necessary but not sufficient for forming a functional complex structure. Moreover, what is required is not merely correct initial morphogenesis, but understanding and implementing reparative robustness in the face of subsequent challenges. Fortunately, the field of developmental and regenerative biology provides extensive proof-of-principle of control circuits that enable efficient self-repair and dynamic control of multicellular, large-scale shape [1a].

Eggs reliably self-assemble into adults with many distinct tissues in precise geometric configuration. Crucially, the embryos of many species are not pre-determined mosaics, but display astonishing capabilities of self-repair, dynamic rescaling, dynamic reconfiguration, and functional plasticity (Figure 1). For example, embryos that are split or combined early in development revise their developmental program to the number of available cells and give rise to multiple *complete* organisms. Dynamic re-scaling of organs allows even adults to incorporate foreign tissue and re-pattern it appropriately; transplanted cockroach legs with the wrong number of segments will undergo intercalation to restore leg segmentation more appropriate to the leg's new location [3], while planarian flatworms continually reconfigure their body tissues to maintain correct relative proportions despite changing cell number during starvation [4].

Adult salamanders regenerate amputated limbs, tails, eyes, jaws, hearts, and portions of the brain; remarkably, the rapid growth that produces these new structures *stops* once the correct pattern has been completed. Moreover, tails ectopically grafted to the flank of an amphibian host slowly remodel into limbs [5], revealing the body's ability to coordinate cell behavior towards a specific anatomical plan. The same remarkable capability is revealed in the process of metamorphosis, as tadpoles will correct experimental rearrangements of their craniofacial structures to reach a normal frog facial anatomy [6]. In all of these cases, the correct shape outcome can be seen as a homeostatic target range; interestingly,

in some species (such as deer antlers, crabs, and planaria), this target anatomy can be re-set permanently [7], revealing that the encoding of the ideal homeostatic anatomical state is somewhat labile and not genetically fixed.

The fact that the process of limb regeneration [8] and embryogenesis [9] can reprogram (normalize) tumor cells into normal structures highlights the causal potency of not only single-cell states but of large-scale anatomical configurations. Development, cancer, and regeneration are distinct processes, which may involve diverse underlying molecular mechanisms in addition to conserved ones. However, what all of these examples have in common is a kind of "shape homeostasis" – the ability of systems to flexibly regulate cell-level events in order to achieve higher-level (organ-, tissue-, or whole-organism) patterning states despite deviations from those states. While recent advances in bio-printing, materials engineering, and scaffolding [10] seek to address creation of complex structures, these technologies do not address the functionality of adaptive (on-demand) remodelling, nor reveal the endogenous biology that allows cellular structures to implement specific morphology changes aimed toward a correct configuration. Here, we define "Target Morphology" as that anatomical state towards which remodelling occurs, and which, when reached, causes a cessation to proliferation and morphogenetic rearrangements.

The major knowledge gap is the understanding of how remodeling of complex shape is driven by the physical activity and information processing of smaller subunits (not necessarily cells). Next-generation bioengineering must move beyond direct assembly of cell types, toward the control of the built-in error correcting morphogenetic networks and the programming of shape by specifying organs and their topological relationships [11]. A key issue for the future of biology and medicine is to find the appropriate theoretical paradigm with which to understand complex pattern regulation besides feed-forward emergence, and derive quantitative models with predictive power that will enable rational modification of shape for engineering and biomedical applications. Here we discuss a complementary, top-down approach, which can encompass the known molecular elements that implement pattern formation: chemical gradients [12], physical forces [13], and bioelectrical signaling [14]. We propose that the field of computational neuroscience has developed theoretical and computational tools that can help understand and exploit pattern regulation as a closed-loop cybernetic system that incorporates feedback mechanisms and operates on high-level (anatomical) metrics (Supplemental Figure 1).

1.2. *A new approach: top-down programming of pattern formation*

Today's dominant approach to pattern regulation is bottom-up – the hope that complex outcomes can be understood via "emergence" once we have all of the relevant details on cellular,

subcellular, and protein interactions. However, it is widely recognized that there remains a gulf between ever finer-resolution analyses of molecular pathways and global understanding of the control of large-scale measurable such as topological arrangement of organs. Indeed, direct management of emergent patterning cascades [2, 11b, 15] is likely to be limited by the inverse problem that plagues complex emergent systems [7]. We hypothesize that efficient programming at the level of anatomical outcome can be achieved if we harness the kind of top-down control algorithms that have been so successfully exploited by nervous systems in the control of animal behavior, and which are studied in the field of computational neuroscience.

The rest of the article is organized as follows. We first discuss one set of control pathways that has recently been implicated in just such a large-scale control of pattern: developmental bioelectricity. Slow changes of resting potential in non-excitable cells regulate the coordination among cells required for morphogenesis, and appear to confer on all tissues capabilities that are usually thought of only in association with neuronal networks. Developmental bioelectricity is of high relevance for regenerative biology because it demonstrates practical applications for exploiting neural-like information processing within somatic structures during morphogenesis. Here, we highlight some features of non-neural cell signaling that can be mapped to information processing in the brain, and elaborate the implications for designing top-down intervention strategies. We next discuss both algorithmic and molecular homologies between information processing in the central nervous system (CNS) and pattern regulation during regeneration development. We propose that shape regulation may be efficiently understood and manipulated as a kind of learning and (constructive) memory/recall process - in analogy to a scheme in which *generative models* learn and memorize patterns and *error-correction mechanisms* trigger actions that involve body changes (e.g., growth and differentiation) that restore them as necessary. In this discussion, we hold to an objective, unambiguous, empirical success criterion for any approach to pattern formation, not an *a priori* commitment to a philosophical position. The best model is the one that optimally facilitates predictable changes in large-scale shape, regardless of whether the model is formulated in terms of genes, information, topological concepts, or anything else (top-down, bottom-up, or mixed). We conjecture that developmental bioelectricity is an emerging field ideally placed to facilitate the practical transfer of insights from computational neuroscience into control of dynamic morphogenesis in biomedicine and bioengineering.

Our goals in this Perspective are to: 1) Refocus the community on the design challenge of programming dynamic, adaptive remodeling capabilities, beyond stem cell differentiation; 2) Review data in developmental bioelectricity, showing how the function of endogenous ionic gradients can be harnessed to implement neural-like information processing in regenerative and bioengineering applications relevant to all cell types; 3) Introduce concepts from computational and cognitive neuroscience, widening the toolbox of bioengineers with new ideas that can be exploited to design

mechanistic strategies for top-down control of growth and form (processes with a goal state, not only bottom-up emergence); and 4) Offer a specific example of a mathematical methodology that can be used to model - and possibly control - pattern formation from a top-down perspective. We synthesize these ideas into a hypothesis about the algorithmic and molecular homologies between information processing in the central nervous system (CNS) and pattern regulation during regeneration development.

2. Harnessing non-neural bioelectricity to implement organ-level programming

2.1. The basics of molecular bioelectricity: definitions and tools

Developmental bioelectricity refers to signaling among non-excitable cells mediated by endogenous electric fields and differences in resting potential [14d, 16]. These bioelectrical states are created by ion channel and pump proteins that maintain voltage gradients across the cell membrane, and are transduced into a variety of transcriptional and epigenetic cell responses by known mechanisms (including neurotransmitter movement) [14e, 17]. Pattern regulation by specific spatial distributions of transmembrane potential (V_{mem}) within tissues has recently been implicated as an instructive factor in numerous patterning events during development, regeneration, and cancer suppression [18], revealing how many cell types exploit the physics of ion flows to communicate much like neurons in the brain, and how this dynamics helps shape complex large-scale morphogenesis. Crucially, as in the CNS, the spatio-temporal patterns of somatic bioelectrical signaling are regulated by flexible electrical synapses known as gap junctions, which establish iso-potential cell regions and maintain dynamic boundaries between compartments with distinct voltage gradients and thus different anatomical fates [19]. It is not surprising that these versatile regulatory building blocks are also implicated in memory, learning, and establishment of circuits in the CNS [20]. McCulloch's answer to why the mind is in the head: "Because there, and only there, are hosts of possible connections to be performed as time and circumstance demand it" [21], in fact applies also to somatic tissues. Highly dynamic changes in selective gap junctional communication and tunneling nanotubes allow any cell field to form complex activity-dependent networks that communicate via electric and neurotransmitter-mediated signaling during pattern formation [19, 22]. Many cell types, including cancer cells [23] and skin [24] cells, are known to propagate gap junction-dependent electrical waves, and GJs regulate global decision-making during the patterning of the somatic left-right axis [25], tumorigenesis [26], head-tail polarity [27], and tail regeneration [28].

Recent advances have resulted in new tools for studying developmental bioelectricity at the molecular level, and for mechanistically linking biophysical events with downstream genetic targets via dissection of transduction machinery [29]. Voltage-responsive fluorescent dyes [30] (Figure 2A,B) and

genetically-encoded voltage reporters [31] allow the monitoring of bioelectric state of complex tissues in vivo. Novel reporters (nano-scale materials that can report physiological parameters via MRI imaging) will even further facilitate the bioelectric profiling of thick and complex tissue, although even today's technology is sufficient to characterize important structures in metazoan patterning models in vivo [30, 32]. Most importantly, a panel of constructs encoding well-characterized gap junctions, ion channels, and pumps enable the targeted modulation of V_{mem} and network topology in any cell group (Figure 3), for functional studies [33]. Mis-expressed or endogenous ion translocator proteins can be regulated pharmacologically, and the technique of optogenetics, which is revolutionizing neuroscience [34], has now been applied to the control of regeneration-specific bioelectric signals [32d, 35].

Suppression screen analysis, in combination with targeted depolarization or hyperpolarization, has shown that slow bioelectrical signals *in vivo* are transduced into downstream changes of transcription and chromatin modification by regulation of calcium and serotonin signaling [32c, 33, 36], just as in neurons. Additional transduction machinery also exists, making use of voltage-gated movement of butyrate [37], voltage-sensitive phosphatases [38], and receptor clustering [39] to convert specific ranges of resting potential (and changes therein) into downstream transcriptional responses and second-messenger signaling events.

2.2. *Bioelectric state controls single cell function*

The V_{mem} state of cells and their neighbors determines cell behaviors, in concert with other signaling modalities. In general, terminally differentiated, quiescent cells tend to be strongly polarized (bearing a more-negative resting potential), while embryonic, stem, and tumor cells tend to be depolarized (closer to zero) [40]. The picture is complicated by the fact that many cells in fact do not have a single V_{mem}, but like neurons bear a set of distinct voltage domains over their surface [41] – analogous to the way an action potential travelling down an axon establishes local domains of depolarization that can underlie computation [42]. While the functional significance of voltage microdomain patterns within single cells (e.g., a combinatorial code of voltage domains on the membrane) has not yet been tested, regulation of overall cell V_{mem} is beginning to be used in bioengineering contexts to regulate cell connectivity [43], wound healing [44], and differentiation [45].

These strategies work because V_{mem} is not a read-out or a house-keeping parameter but is a functional determinant of cell state, such as proliferative capacity, migration, and plasticity [46]. Differentiation and proliferation are controlled by changes in V_{mem}, as has been shown in human mesenchymal stem cells [45a, 47], cardiomyocytes [48], iPSCs [49], vascular muscle [50], embryonic stem cells [51], myoblasts [52], the specification of neurotransmitter types [53], and the precise control of precursor

differentiation [54] in the developing nervous system and heart. Given the known roles of V_{mem} in regulating normal migration, differentiation, and proliferation, it is not surprising that control of bioelectric states is also increasingly implicated in the developmental dysregulation known as cancer [23, 55], and is a suspected causal agent in several kinds of birth defects [56].

2.3. *Bioelectric regulation of large-scale pattern formation*

Most importantly, bioelectric signals also mediate long-range coordinating influences. Spatio-temporal gradients of V_{mem} among cells *in vivo* are now known to regulate organ identity, positional information, size control, and polarity of anatomical axes [14e, 18]. One mode of V_{mem} signaling is as a prepattern. Much like Hox genes, whose combinatorial patterns of gene expression encode specific body regions during development, bioelectric prepatterns in the developing face of the frog and planarian models regulate the gene expression, size, and shape of craniofacial components [57]. In the frog for example (Figure 2B), patterns of hyperpolarization in the nascent face reveal the prospective locations of the eyes and other structures; experimental perturbation of these distributions alters the boundaries of expression of face patterning genes such as *Frizzled*, with the expected effects on craniofacial anatomy. Spatial differences of resting potential can serve as a direct scaffold for subsequent morphogenesis.

Bioelectric gradients also specify orientation of the LR axis in frog and chick embryos [36d, 58] and set the size of regenerating structures in segmented worms, the brain in frog embryos, and regenerating zebrafish tails [32c, 59]. The gradients created by ion transporters, such as the V-ATPase, are required for consistently-oriented left-right patterning of the heart and viscera [36d], fin regeneration [60], and eye development [61]. The instructive information is mediated by bioelectric gradients per se, and not other functions of ion channel proteins or chemical signaling by specific ions: pattern can be predictably altered by specific modulation of those spatial gradients using any convenient channel or ion to achieve the desired change in V_{mem} state [33, 36c]. This offers the opportunity for bioengineers to use structured light (for optogenetic activation) [62] or substrates with embedded channel drugs [63], to impose patterned bioelectrical states on in vitro constructs or regenerating tissues for augmented control of morphogenesis.

In addition to directly specifying the pattern of subsequent anatomy, some bioelectric signals trigger whole developmental modules. In the case of tail regeneration in *Xenopus*, forcing a regeneration-specific bioelectric state in non-regenerative animals for just one hour overcame physiological, chemical, and age-dependent blockade of regenerative capacity to induce complete regrowth of this complex neuromuscular appendage over 8 days [64]. Importantly, a very simple (low information content) and brief stimulus, such as “pump protons”, is sufficient to initiate a complete and

self-limiting cascade of events that rebuilt the entire appendage [16a], in essence providing a "build whatever normally goes here" signal. Likewise, imposition of a bioelectric state via misexpression of specific ion channels can rescue normal brain formation despite the presence of a mutated form of the Notch protein, which otherwise significantly impairs neural development [32c]. These examples reveal that bioelectric state can function as a master regulator, exploiting the innate modularity of developmental cascades; this is consistent with a regenerative medicine strategy which seeks to avoid the need to micromanage morphogenesis of complex structures but rather rely on calling up patterning subroutines already present in the host.

Moreover, bioelectric signals can reprogram the identity of whole somatic regions toward different organs. The morphogenesis of new regeneration blastemas in planaria can be redirected to form heads or tails by imposition of appropriate bioelectric state [36a, 59a]. In vertebrates, whole eye formation can be induced ectopically, far outside the head, even within mesoderm or endoderm tissue (Figure 2C) by misexpression of specific ion channels *in vivo* [33]. This process is mediated by a feedback loop between hyperpolarization and eye genes such as Rx1, but importantly, "master eye inducer" genes such as Pax6 cannot recapitulate this effect (do not induce eyes outside the head in vertebrates), illustrating the benefits of including bioelectric signaling to enhance control over pattern formation. These data reveal that simple stimuli can trigger much more complex, coherent responses (a property that is very familiar to researchers working on memory and hierarchical representation of cognitive content in the brain).

Bioelectric signaling is often not cell-autonomous: cells with unique voltage characteristics serve as organizers, recruiting un-manipulated host tissues to participate in the ectopic morphogenesis (Figure 2D). Bioelectric signaling in normal development, and also in cancer induction [36c] and suppression [37c], is inherently non-local – another property it shares with the way information is distributed within neural networks. For example, during formation of the vertebrate brain, the size of the resulting structure is regulated by bioelectrical information collected from distant regions of the embryo [32c, 59c], implementing a kind of distributed processing also observed during brain function. Much as in the nervous system, electrical circuits in non-spiking somatic cells can coordinate long-range physiological decision-making during pattern regulation.

These examples illustrate the fact that bioelectric signaling provides instructive information to patterning processes by integrating state information across considerable distances, and reveals that morphogenesis can be programmed at the level of complex multi-cellular shape (organs), not only by specifying individual cell types. We suggest that the transformative advances in this field will come not only from ever more-detailed studies of bioelectric signal transduction cascade within individual cells, but will require understanding the bioelectric code: the mapping between dynamics of spatially-distributed

(tissue-scale) bioelectric states and the resulting anatomical outcomes. This parallels neuroscientists' efforts to understand the way that memories and cognitive content are physically represented by the electrical states of brain tissue [65]. The output of somatic bioelectric networks is cellular patterning activity (proliferation, differentiation, migration, and gene expression), much as the output of neural bioelectric networks is muscle contraction and glandular activity.

2.4. *Bioelectricity and non-genetic storage of morphogenetic signals*

The information-bearing signal (the necessary and sufficient trigger) for events such as eye induction, head determination, brain formation, or tail regeneration via V_{mem} change is a *spatially-distributed physiological state*, not a gene product [16]. In many contexts, the exact channel or pump used to trigger such morphological changes is often irrelevant – many sodium, potassium, chloride, or proton conductances can be used to achieve the same morphogenetic outcome as long as the appropriate V_{mem} distribution is enforced [33, 36c, 57]. This means that the actual cause of the given morphological change can be a bioelectrical property not necessarily in 1:1 correspondence with any mRNA or protein. Because channels and pumps can open and close post-translationally, two cells expressing precisely the same mRNA and protein can be in very different bioelectrical states. The cautionary message of these data are that tracking gene expression, and even protein levels, is insufficient – efficient control in regenerative and bioengineering outcomes will necessarily require incorporating sensors and modulators of *in vivo* physiological state. Rich patterns of bioelectrical gradients can exist in a transcriptionally homogenous tissue and be completely invisible to protein and mRNA profiling, precisely in the way that the specific memories of a neural network are not directly visible from a simple survey of which proteins and genes are present. This makes a clear link to the general concept of memory in the information sciences, since engineering models of memory, like electric flip-flop circuits or classic magnetic coil core memory systems, store data in stable energy flow patterns. Indeed, non-neural cells are now known to express ion channel types that implement stable memory elements for discrete voltage states [66], and synthetic bioengineering may exploit as an entirely new kind of memory medium.

One recent set of findings provides an illustration of how bioelectric circuits during regeneration can stably store pattern memory. Planarian flatworms have the remarkable ability to regenerate completely from partial body fragments [67], and the construction of a head or a tail at the correct location in each cut fragment by stem cells is guided in part by an endogenous bioelectric circuit [36a]. Our neural analogy suggests that this information may be stored in the stable modes of the real-time dynamics of a bioelectric circuit implemented by the somatic tissues; if so, then it should be configurable at this same level – our model predicts that it should be possible to stably (permanently) reprogram the basic architecture of the planarian without altering its normal genomic sequence, much as new memories can

be added to a brain without requiring genomic changes specific to each mental state. Indeed, we showed that interfering with the "short term memory", by directly modifying the pattern of resting potentials in the tissue, does indeed allow us to change the tail end of a fragment into a normal head during one round of regeneration [36a, 59a].

Remarkably however, interfering with "long term memory", by altering network connectivity in the planarian fragment (targeting electrical synapses known as gap junctions [20a, 68]), results in fragments developing heads at both ends and *this state is permanent* across future rounds of cutting [27]. Weeks after the initial gap junction-modifying treatment, (Figure 4A-E), when these 2-headed animals have their heads and tails amputated again (in just water, with no further perturbation), the same 2-headed phenotype results, and this is repeated upon subsequent amputations. Thus, a transient perturbation of physiological cell:cell connectivity stably changes the pattern to which the animal regenerates upon damage, despite normal genomic sequence! This phenotype is stable across the animal's usual reproductive mode (fission) – genome sequencing of 2- and 1-headed planaria would reveal no differences, illustrating how patterning information can be stored at the level of a bioelectric circuit. While epigenetic processes may be involved, note that chromatin modification mechanisms alone are not a sufficient explanation since the ectopic heads (tissue which might be suggested to have been epigenetically reprogrammed into a head state from its original tail identity) are *thrown away* at each generation of cutting. What remains is a gut fragment, which somehow knows that it is to form 2 heads, not 1, upon further cutting; what has been changed in such worms is not only the anatomy of one region of the animal, but the encoded pattern that any fragment must rebuild if removed from the body. Such permanent reprogramming of the planarian bodyplan has not been demonstrated using any other method.

The current challenge in this field is to integrate molecular-genetic [69] and anatomical [70] datasets with emerging biophysical models of memory encoded in the bioelectric states of cells [71]. While much remains to be investigated (including tracing the specific patterns of GJ connectivity in living fragments to understand network topology), the planarian example illustrates several important points. First, information functionally determining the large-scale anatomical state of the post-regeneration organism is encoded in the bioelectric signaling among somatic cells. Second, alterations of the bioelectric pattern result in long term, stable changes in the shape memory (similar to synaptic plasticity), while maintaining the organism's same genomic sequence. Third, the information about basic anatomical polarity and body organization must be stored in a distributed form throughout the animal since the altered tissue is discarded at each round of cutting.

These important features of developmental bioelectricity suggest intriguing parallels with information processing in the brain [72] (Table 1). We hypothesize that the basic components of bioelectric

signaling enable neural-like networks in non-neural tissues (Figure 4F-I, Supplemental Fig. 2-3). For example, the distributed storage of information in bodies could parallel the way information is distributed within neural networks [73]. From an evolutionary point of view, this is unsurprising, as neurons evolved by specializing for speed those bioelectrical processes that were already present in far more ancient cell types and being used for morphogenesis [74]. We propose the hypothesis that not only key molecular components of ionic signaling are conserved between neurons and non-excitable cells, but also the algorithms by which they process information. We conjecture that the tricks that brains exploit to guide adaptive behavior evolved from similar computational memory processes that were first evolved to control body patterning, both using some of the same biochemical, electrical, and physiological mechanisms [75].

These parallels with neural information processing are of more than theoretical interest, because in computational neuroscience and cybernetics, practical methods have been developed for pursuing a *top-down approach* to the control of complex hierarchical systems. These data are not only germane to philosophical discussions of levels of control in biology [76], but suggest an empirical, tractable research program for programming shape at a level of organization beyond individual cells. Certainly bioelectric cues do not determine morphogenesis on their own: they represent just one layer of a complex *morphogenetic field*, which guides patterning through interplay with biochemical gradients, transcriptional networks, and materials properties. While a number of these modalities offer cross-scale emergence and long-range control [77], we believe that the available state-of-the-art tools (both technical and conceptual) for understanding electrical networks offer the most tractable approach toward understanding and control of large-scale pattern regulation *in vivo*. Below we discuss in detail a possible top-down approach to pattern formation, using tools from computational neuroscience that are more fully described in the Appendix.

3. A top-down perspective on pattern control

3.1 *Target morphology, error-correction mechanisms, and bioelectrical signals*

Could cell behavior be guided by an algorithm that minimizes - in the cybernetic sense of error-correction - the deviation from a specific *Target Morphology*? We hypothesize that developmental bioelectricity implements true pattern memory. A target morphology could be encoded within tissues using the same kind of mechanisms and algorithms that (learn and) store cognitive memories of shapes and patterns within the brain's bioelectrical network, and underlie directed behavior that seeks to recapitulate encoded goal states.

We propose that models should be explored in which the goal state of anatomical repair is encoded as a true memory of shape, and in which the processes of regeneration make use of recall and decision-making algorithms that parallel those known to occur in neuronal networks. Taking the shape memory analogy seriously immediately leads to two important and testable consequences. First, it suggests that the many tools available to modify memories (from training and behavior shaping to optogenetic inception of memories directly into brains [78]) or to induce plastic changes in somatosensory representations (e.g., extensions of the "body schema" due to training and tool use [79]) could be adapted to developmental bioelectricity to program morphogenesis by re-writing the target states (as in the planarian example above). Second, it suggests that the existing body of knowledge about goal-seeking behavior (from addiction to goal-directed choice circuits) can be mined to create mechanistic but high-level models of how bodies adjust their shape to the same final outcome despite external perturbation. It should be noted that in modeling pattern formation as a primitive cognitive agent, we posit no conscious awareness – merely the same kinds of mechanistic, non-controversial processes that for example allow genetically-specified instincts to guide the spatially-patterned activities of insect behavior.

3.2 *What might a top-down model of target morphology look like?*

We provide an example of a top-down approach to target morphology and morphogenetic fields [80] by using a specific framework developed in computational neuroscience, the Free Energy principle (which is fully described in the Appendix). Note that "free energy" as used here is a mathematical quantity, not to be confused with, say, an animal's metabolic resources or physical properties of its body. Here, minimizing free energy corresponds for an organism to restricting itself to a limited number of "states" that it can occupy, and which are valuable - hence, minimizing free energy roughly corresponds to maximize value. The notions of "state" and "value" are abstract; for example, although an animal might occupy many states (including e.g., being in proximity of a predator) it can enhance its fitness if it restricts itself to valuable states (e.g., being in proximity of food) and roughly correspond to its ecological niche. The free energy principle is thus an attempt to formalize how biological entities maintain their order, and it is now widely adopted in neuroscience, see the Appendix and [81]".

In a Free Energy perspective we cast the growth (or regeneration) of a body part as an *action* - in the sense that it changes the state of the system and can in principle change (lower) its free energy [82]: in other words, an organism can tend to minimize free energy by growing and/or by regenerating body parts. The meat of such a project would be to specify hypotheses for how the system knows the consequences of actions (i.e., acquiring internal generative models), what counts as a state having low free energy (i.e., be a close match of the target morphology), and how are these states coded and memorized (e.g. as *priors*). Here, models and target morphology could be in part genetically determined but also can be acquired during development through self-modeling [83] or somatic surveillance.

To tentatively analyze embryonic development according to the Free Energy rules, suppose that genes predefine some initial *priors*, which describe "good" states of the system (like 'being close to food' in animals); here, equivalently, the "good" states would correspond to some aspects of the target morphology (possibly, without the need to fully specify it), much like the center-boundary structure of the aforementioned primordial soup is not prespecified. The system needs also to specify some initial actions that are available (e.g., chemical messages) to be potentially exploited to achieve "better" states (i.e. with lower free energy). In this context, *growing or regenerating a specific body part is considered as an action* (or a subroutine); such developmental modules are well-known from early experiments with homeotic transformations (HOX genes) and more recent bioelectrical inductions of whole organs [11a, 16a, 84]. Let's assume that initially the free energy of the system is non-zero. Thus according to the usual rule of Active Inference the organism can act to diminish it: *growing*. Although this process is bootstrapped in a genetically pre-specified way, then it is regulated by the usual rules of free energy minimization.

As the body grows, it also implicitly learns the equivalent of generative models (i.e., the effects of specific actions and how they change the free energy) or in other terms it *models its own growth process* (and acquire models that can be potentially reused later on). These models take the form of electrochemical states and they include new *priors* that encode for example the good "target morphologies" (that is, those having low free energy) that are discovered during growth. In other words, while the initial (genetically encoded) priors include some constraints and pre-specify good (adaptive) morphological states for the organism, specific target morphologies are learned or discovered, through self-modeling, during growth. The growth of a healthy body represents a stable solution to the problem of minimizing free energy - in the sense that, when the body is fully grown, it is in a state of low free energy. Any change (damage or aging) actually increases free energy; thus the system tries to counteract this by 'coming back' to its (learned) target morphology.

This speculative model is a framework, one example that entails an answer to the problem of how target morphologies are acquired and then reused as targets (e.g. for regeneration). During embryonic development, free energy minimization guides morphogenesis without a fully specified "stored template" because the template itself has to be created (in the form of priors and generative models). Learning a template might correspond to acquiring new "prior" knowledge on which are "good states" for a system to minimize free energy, and which function as set points within the hierarchical generative

models supporting active inference. However, once a “template” or target morphology has been created that represents a stable solution to the problem of free energy minimization, it can be used to guide remodeling, morphostasis, and regeneration in a top-down manner[1].

In this perspective, the problem of shape regulation is understood as a kind of memory/recall process, where generative models (learn to) encode a pattern or target morphology and error-correction mechanisms trigger actions that restore the pattern. Both the acquisition and the restoration of the target morphology are *active* processes - where specific actions that involve body changes (e.g., growth) obey to the imperative of free energy minimization.

A detailed implementation of this idea is described in Figure 5 and [85]. Here, cell groups self-organize to produce - and successively re-build - a target morphology (a simple form with head, body and tail) under a free energy minimization scheme. In this simplified example, the target morphology itself is not learned - although it could be with some extensions of the model - but assumed to be pre-specified (e.g., genetically). However, there is one aspect of the target morphology that is not genetically encoded but emerges during growth: the cells are initially identical and do not a priori belong to (say) head, both or tail (they all have an identical generative model) see Figure 5A. This means that cells are not "pre-destined" to a unique place but they must undergo a complex epigenetic process and "find their own place" in the morphology - thus, essentially, migrate and differentiate until the whole cell group achieves the target morphology, see Figure 5B. This situation is similar to the dramatic remodeling occurring during planarian regeneration [4, 59a] or the repair of craniofacial defects during frog metamorphosis [6].

The complexity of this epigenetic process emerges when one considers that each cell that tries to find its place influences every other cell by emitting gradients that those other cell sense - thus the population of cells has to find a "collective" solution to the problem [86]. In other words, while a cell "searches" its place in the morphology, it is guided by chemotactic signals continuously emitted by the other (surrounding) cells; but during the "search", it simultaneously emits chemotactic signals that guide

[1] An open research question is how much of the target morphology - and in which form - is genetically specified. Here, there seems to be a significant difference between animals and plants, in the sense that the latter do not generally have a fixed target morphology. This fact leaves open the possibility that, in animals, the (genetic) constraints on the target morphology (or possible morphologies) are stricter; but assessing this possibility deserves future studies. In the simulations presented above, we assume that large portions of the target morphology are prespecified.

the other cells, too. Importantly, the cell's generative model encodes (genetic) beliefs about the chemotactic signals it will (should) express and sense if it occupied a particular place in the target morphology. But even with this information, the cell does not have the guarantee to receive (initially) the "right" signals, or the signals it expects. If all the (other) cells were initially in the right place, and thus emitted the right signals, a cell could find "its" place by simply moving over concentration gradients until it sensed the right signals emitted by the other cells. However, at the beginning of the morphogenesis all the cells are simultaneously trying to find a place. This introduces a sort of circular causality where the cells as a whole concurrently emit and sense signals that influence (and are influenced by) the movements of the other cells; the population has to collectively establish a "chemotactic reference frame" permitting each cell to find its place. A solution to this problem complex (in AI, one would say "multi-agent") problem if one casts the process as the minimization of the free energy of the cell population - because the free energy is minimized when, and only when, every cell occupies a unique target location (see the free energy dynamics in Figure 5B).

It is important to note that engaging in a continuous, dynamical exchange with the environment is essential for the system to maintain its structural and functional integrity and ultimately to guarantee its survival. In the pattern regulation example of Figure 5, "lesioning" the system - that is, preventing cell-cell signaling - leads to various forms of dysmorphogenesis (not shown), consistent with the known patterning changes induced by inhibition of cell communication and movement. Rather, "milder" simulated interventions such as cutting a well-formed animal into two parts induces a dynamic reorganization and regeneration of the target morphology, see Figure 5C.

These examples illustrate how a top-down strategy can tackle an important open problem in biology: how to develop and re-build a "target morphology" and how this produces testable hypotheses, given that all the components can be given a quantitative mathematical specification (see [87] for another example of use of free energy principles to explain shape generation during limb regeneration). The example also illustrates that this top-down perspective is not at odds with useful concepts from (a useful more bottom-up) dynamical systems tradition, such as the notion of emergent self-organization; rather, here self-organization dynamics are contextualized within a general optimization scheme that also makes apparent and permits to predict - for example - under which conditions the perturbations lead to regeneration or dysmorphogenesis.

Some of the tools and modeling approaches for the top-down analysis of pattern formation are already available - often, in other research fields such as computational neuroscience. As a concrete step in the direction of making these tools useful for bioengineers, Figure 6 introduces a formal scheme for the formulation, mathematical analysis and simulation of pattern formation, using the free energy scheme elucidated so far. It emerges from this example that the formal and mathematical methods are in place but at the same time this research agenda requires developing novel quantitative tools; for

example, to quantitatively assess the free energy of a biological system and how the states it can occupy change during growth and regeneration. It is important to note that this is now a very tractable task at the intersection of computational modeling and molecular biology – an area that is now ripe for research.

The free energy principle is just one of the methods that can be used, and several others originating from cybernetics, artificial intelligence, computer science, and control engineering are potentially applicable (see the Appendix). Another example of mathematical approach and top-down methodology (which has not yet been applied to pattern formation, but could be extended to do so) is flux balance analysis [88].

4. Broader implications: homologies between neural information processing and pattern regulation

The example we have discussed is consistent with the broader possibility that deep underlying parallels exist between the way information and cellular control are organized in the CNS and in morphogenesis; this motivates a cross fertilization of methods between these heretofore-disparate disciplines. Below we discuss cognitive-like processes in non-neural structures that underlie pattern regulation.

4.1 *Information processing beyond the CNS*

Concepts formally used to understand cognitive processes in neural tissue may be appropriate to understand regulation of pattern formation. The first requirement is that non-neural cells be able to support basic information processing as occurs in neural assemblies. Indeed, neural-like computation, decision-making, and memory have been reported in sperm [89], amoebae [90], yeast [91], and plants [92], using ubiquitous mechanisms that appear to be also involved in neural information processing, such as cytoskeleton [93] and electrical networks [94]. It is clear that neural networks have no monopoly on such functions, and indeed fascinating examples of memory and neural-like dynamics have been found in bone [95] and heart [96].

4.2 *Neural inputs to pattern formation*

Non-neural tissues perform neural-like functions, while neurons compute using basic mechanisms appropriated from basic cell:cell signaling events. The role of electrical activity in shaping CNS structure is well-established [97]. Not surprisingly, neural outputs impinge on pattern formation in other tissues as well, as the two information-processing systems interface extensively. Examples

include the control of proliferation and differentiation by the signaling dynamics of neural networks [98], the induction of spinal cord regenerative rewiring by electrical activity [99], the mispatterning of deer antler regeneration by neural inputs [100], and the known dependence of regenerating salamander limbs on [101], and addiction to [102], nerves. Importantly, the role of neural inputs in regeneration pattern is not merely permissive, but rather carries instructive information, as revealed by the determination of head vs. tail morphogenesis by the directionality of a transplanted nerve cord [103], the induction of distinct shapes in a regenerated tadpole tail from different locations of damage to the spinal cord very far away from the wound site [104], or the control of seashell patterning by specific neural net output [105].

4.3 *Cognitive concepts in developmental biology*

While a concerted effort to apply neuroscience paradigms in developmental biology has not yet been made, a number of authors have independently used such concepts to help explain pattern regulation. One of the earliest applications explored the extensive parallels between chemical gradients during development and signal processing in the visual system [106], and indeed early quantitative models of patterning (explaining self-regulatory features like proportion regulation) were based on visual system function [107]. More recent efforts include the notion of memory for position during regeneration [108] and development [109], learning models of diabetic electrophysiology in pancreas [110], excitable cortex memory models of pseudopod dynamics [111], and neural network models of chemical signaling [112] (which showed formal isomorphisms between gene regulation networks and Hebbian learning in neural nets) [113]. In addition to classical neuroscience concepts, more exotic group cognition models have been applied to patterning [114], while a few recent studies investigated the decision-making and formal computational capabilities of reaction-diffusion systems – a chemical signaling modality often used to model morphogenesis [115], which is now known to be Turing-complete [116] and to support semantic interpretations [117].

Crucially, cognitive neuroscience research has clearly shown that even high-level mental processes can affect cell growth and differentiation in the brain [118], providing a proof-of-principle roadmap for understanding more broadly how encoded *information* can have causal power in regulating the kinds of cell behaviors that make up morphogenesis.

4.4 *Similarities between morphogenesis and cognition*

In this section, we highlight some of the deep similarities that erode the artificial boundary between brain and body. Importantly, numerous mechanisms are utilized by both – memory/learning and

morphogenesis including connexins, ion channels, neurotransmitters, cAMP, CREB, HDAC, PKA, PKC, mTOR, and many more. Overall it should be noted that many cell types, not just neurons, utilize voltage potentials, calcium dynamics, neurotransmitters, and highly dynamic cell:cell connection patterns to process signals during pattern formation [75a, 119]. Significant recent advances exploiting this overlap include the investigation of neural-like dynamics to explain information processing in plants [120], and the use of non-spiking, slowly changing voltage gradients to model memory [121] in animal systems. This latter effort becomes especially relevant given our proposal, developed below, that non-excitable cells support memory during pattern regulation. It is not often noted that many molecular components of memory, learning, and behavior are also critically involved in morphogenesis. However, nerve cells evolved by specializing much more primitive cell signaling functions first used for development [74a, 122]. Thus, it is likely a true homology - evolution predicts exactly this overlap of mechanisms, making more plausible the idea that undoubtedly-cognitive systems, brains, adapted (and improved) processes that were already being used for primitive cognitive functions during development.

Conservation of molecular mechanisms aside, how are we to understand the encoding of geometric shapes (target morphology for regeneration or development) within biophysical cell properties? This is a fundamental issue that requires integrating very different levels of organization and explanation. An example of this problem in developmental biology is revealed by the fact that depending on cell size, identical kidney tubules can be made of many cells via cell-cell communication or just one cell bending around itself (via intracellular cytoskeletal dynamics) [123]. The requirement of a “3D tube of a specific size” activates very distinct molecular mechanisms to achieve this goal depending on available material (cell size). Understanding such implementation independence requires that we understand how the goal of making a 3D tube of a given size and orientation, which cannot be defined as a single cell or molecular state, is represented as an initiating signal (and later recognized as a stop condition) in vivo. A key aspect of modern cognitive neuroscience is that it provides a roadmap for functionally linking high-level information (e.g., topological shape representations) to molecular level mechanisms occurring in cell networks. Salient examples (Figure 7C-E), with many lessons for bioengineering, include: the alterations of brain cell growth and differentiation by mental practices or spatial learning [124], the insertion of specific (false) memories into the brain by optogenetic modulation of neural cells [78a], and the read-out of mental imagery by processing of brain electrical states [125]. Developmental bioelectricity is a crucial nexus between cognitive science and regenerative biology, which provides an empirically-tractable set of pathways linking higher-order, top-down control and complex system representation and regulation of patterning by cell-level events.

5. Conclusions

5.1. *Summary of new hypothesis: information processing in non-neural bioelectric networks*

We propose that the apparent similarities between concepts in memory/decision-making and regenerative patterning are not merely anthropomorphic ways of speaking about the remarkable robustness of shape control, but underlie real homologies of molecular mechanisms and underlying control logic. Some possible mappings between major concepts in these fields are shown in Table 1. At a mechanistic level, cellular communication models using concepts from neuroscience (synaptic plasticity, long-term potentiation, Hebbian learning, etc.) may be applicable to understanding regenerative control. At a higher conceptual level, we propose that morphogenetic homeostasis may be best manipulated at the level of information processing. By improving cellular recall, and editing memories (specifically changing the stored encoding of a target morphology), as is already being addressed in neuroscience, we may be able to achieve far better control over regenerative processes than we have been able to achieve by micromanaging molecular pathways directly.

Currently, the ability to specify large-scale patterning outcomes is hampered by the difficulty of controlling emergent form by manipulating solely bottom-level molecular events. We propose a complementary strategy, to consider models in which cellular decisions are guided by a process that works to minimize the difference between the current configuration and a "target morphology". Our specific hypothesis concerns one set of tractable molecular mechanisms for implementing top-down control of shape: the encoding of somatic pattern as the semantics of electrical activity outside the brain. Much as developmental modularity greatly enhances the efficiency of evolution [84, 126], subgoaling is a key ingredient of effective real-time cognitive processes [127]; bioelectrical communication and encoding of "subroutine" modules by simpler representations (signals) underlies both, and is thus ripe to be exploited by bioengineering and synthetic morphology applications.

The parallels between neural information processing and regenerative patterning are strong, both at the level of molecular mechanism and of higher-order functions (Table 1). We propose to capitalize on the extensive experience of neuroscience in crossing the level between information (e.g., memories formed during learning or inborn as behavioral instincts) and its physical implementation (synaptic mechanisms and neural circuit dynamics) to address the single biggest question in the field of regeneration: how does an amputated blastema know what shape to make, and how does it know when to stop growing? Recent data implicate bioelectrical signaling in non-neural cells as a major regulator or large-scale anatomy, and show that the differences between neural and non-neural cells are not fundamental: all cells make networks with highly tunable electric synapses, and propagate signals via voltage dynamics and neurotransmitter signaling. It is likely that processing in the brain is a highly-accelerated version of basic cell mechanisms that existed long before a fast CNS was evolved for motile

behavior; indeed, the computational abilities of astrocytes may be an intermediate case [128]; the role of non-spiking cells such as astrocytes and glia in memory and cognition [129] reveal that the brain already knows how to process information in non-excitable cells. Developmental bioelectricity is thus the most likely physical mechanism for implementing top-down, goal-driven processes that might regulate pattern formation. More specifically, we propose that representation of anatomical goal states within bioelectric circuits of somatic tissue is a true kind of memory, both in terms of its conserved molecular mechanisms and in the algorithms through which it operates. Importantly, boelectricity is not the only signalling modality consistent with this approach; for example, Hox gene expression patterns "constitute a form of positional memory – an internal representation by a cell of where it is located within a multicellular organism" [130].

In a sense, the current state of bioengineering is a kind of behaviorism, which ignores internal information representation and goal states and speaks only of cellular or molecular behaviors. Much as behaviorism was supplanted by a more powerful and empirically-successful theory of cognitive neuroscience (which exploits the reality of multi-level semantics, goal states, and information processing in the CNS), we argue that the next steps of biological control will involve taming the representation of patterning states within tissues. In this new strategy, bioengineers will seek to exert control by hijacking these bioelectrical pathways to rewrite the shape descriptor to which cells are working, and thus program pattern to an organ-level specification. Paralleling the development of cognitive science, we propose a kind of Intentional Stance towards models in this field, which focuses on extent of empirical control of shape, over a priori commitments to the form that such models must have (e.g., molecular pathways).

5.2. *Next steps and transformative opportunities*

There is little doubt that current approaches will continue to reveal molecular details of bioelectric signaling within cells. What will require out-of-the-box (interdisciplinary) thinking is the understanding of the bioelectric code: the mapping of distributed V_{mem} states to specific anatomical outcomes. How best to quantitatively model the circuit dynamics and resulting stable attractor states that orchestrate individual cell activity into maintenance of specific large-scale states? We have at least one example of a successful research program in which high-level semantics are being merged with molecular-level mechanisms: computational neuroscience; consideration of its deep insights could strongly enrich understanding of developmental biology.

Our hypothesis is testable, and suggests a rich research program. Specifically: (1) the development of improved methods for reading/writing bioelectrical state information into somatic tissues and sculpting non-neural bioelectric circuits (advances in optogenetics beyond excitable cells and in the

synthetic biology of gap junction and neurotransmitter signaling) [32d], (2) continued work on cracking the bioelectric code (understanding how bioelectric state information maps onto the topology of various patterning outcomes in tractable model systems such as planaria) [16a], (3) formulation and testing of quantitative, molecular models of LTP, habituation, sensitization, and synaptic plasticity applied to slow bioelectric signaling in non-neural cell groups regulating regenerative growth [96b], (4) use of reagents that impact cognition (hallucinogens, anesthetics [131], stimulants, nootropics/cognitive enhancers, etc.) in developmental and regenerative patterning assays to probe conservation of pathways between neuroscience and morphogenesis, (5) *in silico* study and synthetic implementation of biophysics models of circuits which can stably store bioelectric state information as attractor states of ion channel activity in arbitrary cell types [132], (6) creation of larger-scale computational models of regeneration and functional experiments in morphogenesis based on goal-seeking and error minimization algorithms with molecularly-specified metrics [133], (7) exploration of molecular models of cognitive concepts (attention, autism spectrum, sleep, visual illusions/hallucinations, addiction) in specific patterning and mispatterning contexts, (8) experimental examination of learning and complex behavior [134] in non-neural *in vitro* constructs to understand the cognitive powers of non-excitable cell networks [135], (9) bioengineering platforms that reward and punish in vitro patterning systems for specific changes in growth and morphogenesis (seeking to demonstrate instrumental learning and top-down control of shape in developmental or regenerative contexts), and (10) a mechanistic investigation of the mechanism of persistence of memories through massive brain regeneration, which is likely to reveal the interface between somatic and neural memories [136].

5.3. *Broader outlook*

We propose taking seriously the idea that patterning systems may be, in a mechanistic and algorithmic sense, primitive cognitive agents that remember specific shape configurations. One immediately tractable way to test these ideas is through mapping the bioelectric code; this way of tackling pattern regulation could provide empirically efficient control of biological shape for regenerative biologists and bioengineers. Top-down models may facilitate altering encoded goal states (e.g., target morphologies), bypassing the complexity explosion currently facing regenerative medicine's attempts to control complex shape by tweaking molecules. It may be possible to efficiently "train" morphogenetic systems to desired outcomes, by providing rewards (or "objective functions") for specific outcomes instead of micromanaging the underlying signaling. Likewise, a better understanding of the bioelectric code may allow optogenetic or similar methods to rewrite the target morphology in vivo, inducing cells to build desired patterns as a kind of universal constructor. Interestingly, this effort may also pay off in the reverse direction, shedding light on the semantics of bioelectric states in the brain. However, cybernetic

strategies are applicable to top-down regulation via any mechanism, not only bioelectricity, and can readily be explored in the context of biomechanical forces, gene regulatory networks, etc. For example, an area to be investigated is the application of active inference models to gene-regulatory networks and protein interaction networks [137], attempting to analyze their dynamics as an information-processing structure.

There is no doubt that for some systems, bottom-up emergence is a powerful framework [138]. We think it is also essential systematically explore the other side of the coin for those areas where complexity limits the efficiency of explanations at purely the molecular level [7]. Computer engineering and neuroscience serve as proofs-of-principle that efficient control of complex systems can be pursued with top-down models of goal-directed activity. Concepts such as feedback control and goal-seeking algorithms must also be included in training courses that nowadays focus principally on differential equations for gradients and network analysis, omitting complementary perspectives from computer science and engineering despite the ubiquitous calls for a deeper integration across disciplines. Ultimately, it is an empirical question whether a given biological phenomenon is better addressed from a bottom-up, top-down, or combined perspective. Training young scientists in both approaches will permit them to exploit the remarkable opportunities revealed by the dynamic capabilities of pattern regulation, and reap the benefits of achieving complete control over growth and form.

Appendix 1 (Supplement). Harnessing top-down controls in cognitive systems

Bioengineering must explore not only the molecular tools and cell-level models of computational neuroscience (Section 3 below), but also the conceptual frameworks for analyzing top-down controls in biological systems. This section introduces the bioengineer to a few such paradigms, successfully used in other sciences, which may find application in understanding and controlling the high-level robustness of pattern regulation. The subsequent section presents a specific detailed proposal for cracking the bioelectric code, based on the insights of neuroscience, in which bioelectric states of somatic tissues encode memory patterns to which growth and morphogenesis operate.

A1.1 *Beyond teleology: goal-seeking mechanisms*

Teleology is the claim that some biological process is proceeding towards a pre-specified goal, and is an obvious first thought for any student encountering embryogenesis or regeneration. However, goal-directed processes do not imply magic: they are widely accepted in cybernetics, computer science and computational neuroscience, which are replete with systems that try to achieve a state somehow encoded, stored, or remembered. A brain (or other physical control structure) represents future goal states and triggers behaviors (or inferential processes) that minimize the distance between the current and goal states [139]. Goal states can be explicit such as "reaching location X" or more implicit such as in homeostatic systems that seek to maintain interoceptive variables (linked e.g. to thirst or hunger) within a safe range.

The importance of control mechanisms and of error-correction mechanisms can be traced back to evolutionary demands. For all organisms - simple or complex - to remain living, certain conditions required for their proper operations must be met. The teleological strategy assumes that organisms are not passive but take an active part in ensuring that these conditions are met via *control* of variables such as temperature or nutrient levels to acceptable (homeostatic) ranges using monitoring, error-correction mechanisms and actions such as locomotion and ingestion. Homeostatic control has been often described in terms of negative feedback-based mechanisms that continuously monitor internal variables and trigger actions (e.g. food seeking) to keep them within acceptable ranges. Error-correction mechanisms in higher animals such as primates can be much more sophisticated; one example is the control of human reaching movements in which action is controlled towards some specific goal location (e.g., a food location) rather than being random, and internal forward models support feedback mechanisms and permit compensating for sensory delays and uncertainty [140]. Another example is goal-directed rodent navigation and planning. Neural recordings in the hippocampus show that during pauses at decision points the animals can "mentally simulate" future spatial trajectories to select among them and plan how to reach a given goal location [141]. These are examples of deliberative forms of decision-

making, where an animal "fills the gap" between its current and the desired locations (e.g., a food location) firstly in its own brain, and only successively takes action. These examples illustrate a continuum of goal-oriented or goal-seeking cybernetic mechanisms, from those permitting to follow temperature or sugar gradients to those permitting to achieve distal goal states in humans or other animals. These goal-oriented mechanisms, carried out by cellular networks in the brain, implement progressively simpler-to-more-complex teleological mechanisms.

A prototype of teleological mechanism in cognitive science is the *test-operate-test-exit* (TOTE) cybernetic model [142] (Figure 7A). In a TOTE unit the first operation (test) is testing if a goal state has been achieved (a form of feedback control). If not an operation is executed and the test is performed again; this sequence can be repeated until the test on the goal achievement is successful (and then the TOTE unit exits). Consider for example the task of hammering a nail. The test consists of verifying whether the nail's head touches the surface; the operation consists of hammering the nail until the test condition holds. This simple goal-seeking mechanism can be extended to more sophisticated aspects of teleology and goal achievement such as the ability to predict action consequences and to plan multiple steps in advance [141] if one also includes in the scheme internal generative models - a point to which we will return later. Furthermore, goal states need not to be static but can be dynamically set by higher levels in hierarchical control systems [143].

Engineering routinely uses feedback controllers (e.g., a car's cruise control). The desired value of a reference variable (e.g., a certain position or speed) is compared with the actual variable value; the discrepancy serves as an "error signal" that is used to adjust the system's position or speed. Given its robustness and reliability, in several practical circumstances (e.g., in control engineering set-ups) this simple feedback-control mechanism (with various extensions) is preferred to alternative controllers that only use local rules, e.g., encode responses to environmental stimuli, and it is reasonable to expect that evolution exploited this strategy. When severe craniofacial mispatterning is induced in tadpoles, correct final facial pattern in froglets is nevertheless achieved by dynamic reconfiguration of organs: the resulting adult frogs exhibit normal faces, showing that their genome specifies not invariant movements for the various facial components but a complex and flexible process that is applicable to many starting configurations and can achieve a correct target morphology via one of many different paths [6]. This can readily be modeled using a TOTE-like concept (Figure 7B). What is encoded is not a hardwired set of movements for turning a normal tadpole face into a normal frog face, but rather may be the target state of the final product (the correct frog face shape) and a mechanism enabling cells to move to minimize the difference between their current configuration and the target morphology. Such a model explains the remarkably plastic ability of the tissues to carry out the necessary movements and then stop when the pattern has been achieved, despite an experimental perturbation that could not have been predicted in detail by evolution. Indeed, in any example of regenerative repair, this type of model focuses on representation of correct state and deviations from that state (high-level properties), while current models

exclusively focus on molecular events in the hope that anatomical repair appears as an emergent property. Note that no goal is being ascribed to evolutionary processes, but rather to cellular reconfigurations within an organism.

To make use of a top-down model in regenerative medicine (activate error-correction mechanisms and “repair towards correct target morphology” in any desired organ), we must ask how cell groups store specific patterns (memories of the correct state, internal generative models) and how they ascertain current state as deviations from the target morphology (monitoring mechanisms). In keeping with the evolutionary conservation of major pathways, it is reasonable to look for such mechanisms within the brain – a system optimized for storing pattern information and detecting differences between global features, at multiple levels and timescales. The discussion of generative models and deep networks below will clarify how such learning mechanisms work, and hopefully spur novel efforts to build testable models of this type for developmental patterning.

A1.2 *Control theory and internal models*

Several scientific disciplines study (and also build) teleological systems, including control and systems engineering, Artificial Intelligence, cybernetics, and computational neuroscience. Already von Neumann in the 50’s asked what are the requirements for a machine that self-replicates and answered in terms of control-theoretic and information principles, highlighting the importance of instructions (e.g., in the DNA), a duplicator, and a controller [144]. More recently these disciplines have developed formal concepts and computational systems that can be potentially used to study morphogenesis from a top-down perspective.

The notion of "control" is central in the cybernetic study of systems including living organisms. A controlled system is one that regulates a given variable (say its position) based on a set point or reference value (say a desired position corresponding to e.g., a food place). The "control loops" include sensors and actuators, but also control algorithms that can vary in their complexity. Simpler control systems use only (negative) feedback for regulation, while more complex controllers also include *internal models* [145]. In cybernetics, the Good Regulator theorem [146] states that "Every Good Regulator of a system must be a model of that system" (which has interesting implications for pathways that regulate morphogenesis). In computational motor control, internal models have (at least) two prominent roles: computing the necessary actions or motor commands to achieve a certain goal given a starting condition (inverse modeling), and predicting the sensory consequences of those actions (forward modeling) [147]. For this, the models themselves encode (probabilistic and possibly hierarchical) information on how sensations change over time, and particularly under the influences of actions. These concepts set the stage for thinking about developing and regenerating structures as agents that represent (model) their current and/or target shapes in some physical encoding as they make decisions that guide differentiation, movement, and physiological signaling.

Internal models encode the dynamics of a given system and can be used to reproduce the same dynamics when fed with the appropriate input. The input needs not to come from perception but can also be self-generated. In this vein, it has been hypothesized that internal models can be used for imagery and for mentally simulating or planning a given action sequence; the imagined actions provide fake feedback that is used to continue the mental simulation without overt perception and action [148]. A recent trend in computational and systems neuroscience is that of unifying several constructs of control theory and internal modeling in terms of probabilistic (Bayesian) inference. Here the general idea is that any control problem can be equivalently cast as a (probabilistic) inference problem [149], where usually the variables to be inferred are the control states that permit a transition from the current state to a goal state [81, 150]. For example, one can see a control problem in terms of the reduction of the discrepancy between (probabilistic representations of) a start and a goal state, and use probabilistic computations to infer the best sequence of actions minimizing this discrepancy (as in for example KL control [151] or Active Inference [152]). In turn, this requires the agent to possess internal (generative) models of sensations and actions of the kind described earlier, and to encode the desired (goal) state as a target remembered state (e.g., a prior probability) within the models.

One example that suggests such internal models and priors is the phenomenon of trophic memory in some kinds of deer (reviewed in [7]). Each year, these animals shed their antlers and regenerate a rack of the same morphology. Remarkably, if a wound is made at one point within this branched structure, for the next ~5 years, an ectopic tine will be formed *at the same location* in the new rack. Since the whole structure is replaced each year, this requires that the cells in the scalp remember the location of the damage (in 3D space or within some other more compact encoding of the branch points) and use it to guide the behavior of cells as they recreate the antler pattern each year to include an extra branch at the correct location. This phenomenon suggests that the structure of the antlers is represented in some way within the remaining scalp cells; this data structure can be modified (e.g., by wounding) and guides next year's cell proliferation and differentiation to form a distinct shape [7].

In principle, internal models can be used in any application. Internal modeling methods can also be used for self-modeling: to model and infer one's own structure (e.g., body morphology) and to correct structural changes (e.g., recover from body changes). For example, robots can build models of their own structure that permit predicting the sensory consequences of its movements and use it to maintain its integrity despite injuries [83]. It is a testable hypothesis that known examples of adaptive behavior following significant body reconfiguration [153] likewise rely on a period of self-exploration.

A1.3 *Generative models and Deep Learning*

One key aspect of the control and internal generative models introduced so far is how they are acquired or learned in the first place. In developmental biology, the target morphology can be hardwired (e.g., genetically specified), or derived from *dynamic surveillance* of current shape (e.g., in regenerative

systems that display trophic memory, such as deer antlers, crab claws, and planaria [7]). A traditional distinction in machine learning is between *supervised* and *unsupervised* learning methods; the former abstracts patterns from observing pre-sorted or "labelled" examples of a given class, while the latter automatically finds pattern in data (builds representations that reflect statistical structure within the inputs).

A particular class of connectionist unsupervised methods, called *deep networks*, are especially successful in current machine learning [154]. Some deep nets - the *deep belief networks* (DBNs) - are *generative* neural networks that have two or more layers of neurons, with connections between the layers but not between units within each layer. Usually, within DBNs one layer serves both as input and output, and one or more internal (hidden) layers acquires increasingly more abstract models of the input data [155]. These systems encode a (usually probabilistic) model of how the data are generated (i.e., a joint probability distribution over data and hypotheses or models). When trained using sets of examples, DBNs can learn to probabilistically reconstruct their inputs. During such unsupervised training, inputs (e.g., figures of animals) are fed to the input layer. The network's task is simply reproducing (or "generating" or "hallucinating") the same figure as in the input layer using the joint probability distribution. Learning consists of iteratively reducing prediction errors: the discrepancy between the "generated" and "sensed" inputs by adjusting the bidirectional weights linking the input and hidden layers. Eventually several hidden layers can be "stacked" that encode increasingly more profound and abstract regularities; for the visual task we have described, this mechanism could mimic the brain's visual hierarchy which extracts key anatomical features. (In machine learning, usually the top layer of DBNs is also trained in a supervised way to perform classification of the input categories, e.g., of animal types.)

A1.5 *Predictive Coding, Active Inference and the Free Energy principle*

Another, related class of generative models that is gaining prominence in computational neuroscience is that of "Predictive Coding" architectures, which use the minimization of prediction errors between internally generated and sensed inputs for perceptual inference - an idea that dates back to Helmholtz [156]. A predictive coding architecture consisting of two (or more) layers encodes a probabilistic model of the causal dependencies between "perceptual hypotheses" expressed at the higher layers and sensory data expressed at the lowest layer. Inference consists of reconstructing the hidden causes of the observed data (e.g., is the object I see in front of me a cube or a sphere?). During perceptual inference, the higher layer encodes perceptual hypotheses on the possible causes of the inputs (e.g., the cube or the sphere). It tries to predict the input encoded in the lower layer (e.g., the visual appearance of the object in front of me). In turn, the lower layer sends back prediction errors that permit revising the initial hypothesis (say, pass from the cube hypothesis to the sphere hypothesis). By iterating this process of top-down and bottom-up message passing, an "agreement" is formed so that the hypothesis which explains better the inputs wins - where, importantly, the impact of both predictions and prediction errors

in the inference is modulated by their respective precision (or inverse variance). In this situation prediction error is minimized because the higher level does very accurate predictions [157]. This model of perception consists thus in predicting the causes of sensory inputs so as to minimize prediction errors.

The *Free Energy principle* [81] is a general theory of brain organization and processing that generalizes the predictive coding idea in several ways. It proposes the minimization of *free energy* as a generalization of prediction error that can be extended to multiple hierarchical layers. It also proposes that free energy (or prediction error) can be minimized in two ways: by revising perceptual hypotheses, and by *acting*. In the cube vs. sphere example, another way to minimize prediction error generated by the cube hypothesis is presenting or putting a cube in front of the subject - this extension of predictive coding to action is called Active Inference [81]. Importantly, in the Active Inference scheme the representations at the higher levels play the role of goal states (e.g. I want to see a cube in front of me) rather than just perceptual hypotheses. Using Active Inference, the architecture can achieve its goals in an open-ended way just minimizing free energy and the discrepancy between current and goal states through action (Figure 8). Of note, here the internal generative models can be hierarchical (or *deep*) and encode regularities at multiple time scales (e.g., short- and long-term predictions of how sensory states evolve over time as an effect of actions or action sequences). By "inverting" the models, the Active Inference schemes can plan, that is, compute the action sequence that leads to distal (goal) outcomes, which are the states that minimize the free energy of the system. Here, a circular causality is evident in the system because the goal states are both *priors* (and thus causes) and *consequences* of action - where the apparent contradiction is resolved by noticing that goal states play these two distinct roles at two different moments in time, before and after an action takes place. The Active Inference view is related to the TOTE idea and cybernetic models more generally [142, 158] and has a clear neuronal implementation in the brain [81].

The Free Energy framework addresses learning processes in a manner that is similar to deep learning. Briefly, the brain progressively acquires generative models that encode the statistical structure of (or “maximize evidence about") the environment in which they are immersed (e.g., encoding regularities in the sensorium, the rules that regulate body movements and their consequences) - this can be considered as a form of Bayesian model selection of the kind adopted in many data analyses. From a more biological perspective, during this learning process, new *priors* and high-level hypotheses are formed that encode potentially high-value goals (e.g., earning money) that correspond to states where free energy can be minimized. These complex goals derive from simpler, genetically specified goals (e.g., find shelter and food) but afford more sophisticated adaptive behaviors; the novel priors go hand-in-hand with the newly acquired generative models that describe how to use actions to earn money [159].

The Free Energy framework extends beyond the domain of computational neuroscience. Friston [160] provides a description of generative models in biological systems at large; for example, in a "primordial soup" of elements (e.g., cells): dynamical subsystems that are characterized by their own

structural and functional states but are also coupled through short-range interactions (Figure 8B). In this context, a "generative model" can correspond for example to electrochemical states (e.g., concentrations of specific signaling molecules in a cell) and the "actions" correspond for example to chemical messages, which determine changes in electrochemical states of e.g., other cells, and the whole process is guided by the imperative of *free energy minimization*. The appeal of this formulation is that it becomes possible to study in a top-down manner the ensemble dynamics of the system, such as the self-organization of a center-boundary structure or the way a "primordial soup" models its environment and acts on it to preserve its structural integrity and resist the second law of thermodynamics. This example shows how top-down concepts (from hierarchical probabilistic inference) and bottom-up concepts (dynamical systems and emergent properties) can be combined to study information flow and pattern self-organization in biological systems [137b].

The concepts of control, generative models and goal states constitute a toolbox for building biological theories and generating empirically-testable hypotheses. The use of top-down methods and concepts can have benefits both when they are used alone and synergistically with bottom-up analyses such as cellular automata models [161]. For example, the Free Energy principle retains essential benefits of "emergentist" theories (e.g., diffusion mechanisms or cellular automata experiments [161]) in that it emphasizes self-organization and only requires local information transmissions ('message passing' between neurons or cells). At the same time it is a normative theory and specifies a global objective function (minimizing free energy) that prescribes the system dynamics, rather than only describing local rules; and it provides a mathematical characterization of learning processes that can be used to study growth (and regeneration) processes - and also in principle to influence them causally.

In the main text, we have sketched an initial illustrative proposal of Free Energy that could be adapted to study how a biological system can first "discover" a target morphology during epigenesis, and then use it as a "set point" for regeneration. Other applications, such as to immune system function or inflammatory cascade signaling networks, are certainly possible and remain to be investigated.

A1.4 *Examples of additional concepts in the top-down toolbox*

In focusing on top-down mechanisms, we are not saying that the molecular details are unimportant, but only that we cannot take for granted that the best model in bioengineering must be at the level of protein interaction (vs. atomic forces, or anatomical topology). In physics and engineering there are many successful examples of concepts described using a coarse-grained approach, which purposely abandons the tracking of individual components in favor of ensemble properties that take center stage for manipulation and system analysis. One example is the Boltzmann definition of entropy that captures the statistical properties of a system composed of myriads of elements, rather than tracking the behavior of the individual elements. In thermodynamics, not only entropy but also several other macroscopic variables such as temperature or pressure describe the average behavior of a large

number of microscopic elements. Statistical methods permit linking macro- and the micro-levels and characterizing causal relations between them, in both a bottom-up (i.e., a given temperature results from specific kinds of microscopic dynamics) and top-down way (i.e., raising the temperature of an object has cascading effects on its micro-constituents). In this latter example, one can establish rules that govern the system but depend on concepts and "control parameters" that exist at levels higher than the micro-components [76g]. This idea meshes well with the concept of an emergent property of a system. Note that here emergent properties are not just by-products to be measured (outputs), but they can actually be controlled to change the behavior of the system (inputs).

Attractors dynamics have been widely studied in physics and since the pioneering work of Hopfield [162], there is a flourishing literature that uses this concept to model semantics and computation implemented by neuronal dynamics. The benefit of attractors is that they illustrate how a mechanistic system can evolve *toward* a stable state or set of states. Furthermore, the concept of an attractor is a general high-level descriptor of bottom-up self-organization processes; if the system reaches an attractor basin, certain specific details are not required to understand its behavior (e.g., it goes towards the basin of attraction regardless of its initial state). The concept of attractors as causal factors in networks is currently being explored in cancer and synthetic biology applications [163]. Moreover, hybrid approaches have been developed that combine control engineering and dynamical systems; for example, by designing individual components (e.g. cells or their components) as controllers having a specifically designed function, but letting their interactions emerge through self-organization and distributed computation [164]. Finally, attractor dynamics can be used within the active inference scheme introduced earlier, where for example sequences of attractors (forming a *stable heteroclinic channel*) become part of the agent's generative model and guide sequential behavior and the transitions between motor acts [165].

Another key concept in the top-down toolbox of many sciences is that of *information*. Models in which information transfer plays a central role have been developed - for example - in artificial life and cognitive science [166]. Robotic control systems have been realized that are able to autonomously learn an increasingly sophisticated repertoire of skills by iteratively maximizing information measures, such as for example their *empowerment*: roughly, the number of actions an agent can do in the environment or its "potential for control", as measured by considering how much Shannon information actions "inject" into the environment and the sensors [167]. Empowerment or related information measures such as predictive information, homeokinesis, and others [168] can provide universal metrics of progress of agents' perceptual-motor capabilities and permit them to learn new skills without pre-specified learning goals (e.g., learn this or that). Furthermore, informational measures can be used to realize algorithms that plan and control behaviour using less information resources, thus yielding parsimony in inference and control [127]. Information-theoretic analyses have been used to model how animals restore their homeostasis in a teleonomic way from both metabolic and informational points of view [169]. Measures of information integration and entropy have been also adopted to study brain networks and even to develop a measure

of consciousness by casting it in terms of highly integrated information processing in the brain [170]. Along with other methodologies to study complex brain networks [171], these measures can be potentially used to study networks of non-neural cells.

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Figure Legends

Figure 1: Examples of dynamic pattern regulation

Large-scale patterning during regeneration and embryogenesis often exhibits flexible growth programs that work to achieve a specific target morphology. (A) Embryos of many species can be split in half but result in two perfectly normal individuals – monozygotic twins (photo by Oudeschool via Wikimedia Commons). (B) Similarly, mouse embryos can be joined together and yet re-pattern to give rise to a normal animal. (C) Salamander limbs can regenerate perfectly following amputation, and the process stops when a correct limb is rebuilt. (D) A tail grafted onto a flank of an amphibian slowly remodels into a limb – a structure more appropriate to its new anatomical position; this includes re-specification of the distal-most tip into fingers, showing that the process is non-local (because the immediate environment of the tail tip is its expected "tail" context, and it should have no reason to change unless it received long-range signals). (E) In some species of deer, damage at a particular spot on the invariant branched structure will result in an ectopic tine appearing *in that same location next year* after the antlers are shed and re-grow (used with permission [172]). (F) A tadpole modified during development such that its craniofacial organs are in the wrong positions nevertheless develops into a normal frog, showing the ability of morphogenesis to flexibly correct unexpected initial states towards the same anatomical outcome (frog image courtesy of Erin Switzer; tadpole image used with permission [6]). These examples illustrate the ability of biological systems to robustly pursue or maintain a goal state specified at the level of topological arrangement of organs – a capability we must learn to exploit, for transformative applications in synthetic bioengineering. We do not discuss plants, because though they often possess impressive powers of regeneration [173], they generally have no fixed target morphology at the level of the entire organism. Images in panel F are courtesy of Douglas Blackiston and Erin Switzer.

Figure 2: Non-neural cells use bioelectrical signaling for pattern formation

(A) Voltage-reporting fluorescent dyes reveal a rich pattern of bioelectrical communication among early frog embryo cells. (B) During later development in the frog embryo, a prepattern of hyperpolarization is seen (lighter cells) which establishes the prospective boundaries of craniofacial gene expression and the location of anatomical organs: in this way, bioelectric state information directly and functionally encodes the anatomy and structure of the face (used with permission [57]). If this bioelectric pattern is artificially perturbed, predictable changes in face morphology result. (C) Targeted changes of bioelectric state, by misexpression of ion channel mRNA in frog embryos in vivo, reprogram body regions at the level of organs: without having to specify the details, a portion of the gut can be re-

specified to form a complete eye (red arrowhead; used with permission from [33]). (D) The process involves not only the cells whose voltage properties were changed (marked with blue lineage dye) but also recruits some of the host's unaltered cells toward making a complete circular lens, revealing a non-local property of bioelectric organ induction.

Figure 3: Tools for perturbing bioelectrical networks

Much as in the nervous system, there are 2 basic options for experimentally modulating the activity of bioelectric networks in developmental contexts. Analogous to synaptic plasticity, the connectivity of the network can be modified, by blocking endogenous gap junctions (electrical synapses), either pharmacologically or via misexpression of a dominant negative connexin subunit, or introducing novel gap junctional connections by driving expression of wild-type connexins or connexin mutants with desired gating/permeability properties. Analogous to intrinsic plasticity, one can instead modify directly the bioelectrical state of specific cells. Pharmacological, genetically-encoded, or optogenetic strategies can be used to modify which channels are expressed in cells, or which are open/closed. Guided by the Goldman equation, these interventions can be designed to result in desired changes of resting potential in the targeted cells. Images in this figure were created by Jeremy Guay of Peregrine Creative.

Figure 4: Pattern memory encoded in bioelectric circuits

Planaria (A) can regenerate any body region, and their head-tail polarity is regulated in part by an endogenous voltage gradient. When the head and tail are removed and the middle fragment is treated with reagents that alter the topology of the bioelectric network (gene-specific RNAi targeting innexin proteins, or gap junction-targeting drugs that wash out in 24 hours, B), a 2-headed planarian results (C). Remarkably, weeks later, when these animals are cut and re-cut in plain water, 2-head worms continue to result (D,E) despite the animal's normal genome and the fact that "epigenetically reprogrammed" tissues are removed at each round of cutting. This illustrates the distributed encoding of target morphology among all body regions, the storage of pattern information in bioelectrical properties distinct from genomic information, and the ability to alter the shape to which this animal repairs upon damage by changing network connectivity among cells long-term memory (all ideally mirrored by the known properties of long-term memory). Bioelectric circuits that could stably store such state information consist, much like neurons, of voltage potentials driven by ion channels (F, transcriptional changes in the expression of which are analogous to intrinsic plasticity in neuroscience) and of connectivity via highly tunable electric synapses – gap junctions (G, changes in which are analogous to synaptic plasticity). (H) Positive feedback loops between voltage states (an aggregate, systems property) and voltage-sensitive ion channel states allow stable attractors of distinct bioelectrical states. Together with known

mechanisms of synaptic plasticity implemented by gap junctions, calcium, and neurotransmitters (I), these components should allow the creation of mechanistic models of pattern memory and the construction of synthetic bioengineered devices with memory and self-repair capabilities. Panels A-E used with permission [18]. Images in panels F and G were created by Jeremy Guay of Peregrine Creative. Images in panel H used with permission [75b].

Figure 5: Pattern formation and regeneration using the free energy principle (FEP)

(A) A sample computational model [85], in which undifferentiated cells self-organize to reach a target morphology, corresponding to a (simple) multicellular animal with head, body, and tail (e.g., a planarian). The target morphology is specified in such a way that, when it is achieved, all cells essentially sense the “right” electrochemical signals – a state in which no further remodeling (cellular activity) is necessary. However, the problem for the cells is "finding their place" in this target morphology; because cells are initially undifferentiated, each can (in principle) become part of the head, body, or tail. This morphogenetic process is formulated as an inferential, FEP problem (B), where essentially the whole system undergoes a series of changes (e.g., in cellular position) until the target morphology is achieved. While changing their place, cells emit signals (chemical and/or physiological) that in turn guide the other cells, until a collective solution is found that corresponds to the state where the free energy of the whole system is minimized. Once the system has reached a stable solution, it can be perturbed, e.g., cut into two parts, (C) and this can lead to a new morphogenetic process with the regeneration of two organisms. Perturbing the system in more severe ways can lead to various forms of dysmorphogenesis (not shown, see [85]). Note that this self-organizing process is guided by an objective function (free energy minimization) and lends itself to top-down analysis, while able to accommodate known details of cellular signaling. Images reused according to the Creative Commons license from references [85, 160].

Figure 6: Formulating and solving a patterning problem via the free energy principle (FEP)

The figure schematizes a "methodological recipe" for formulating and solving a patterning problem using the free energy principle (FEP); see [85] for one recent example where this approach has been successfully used. The methodology is composed of three steps. The first step (A) requires specifying mathematically the so-called generative model of the cells, or in other words their "internal states", "active states", "sensory states" and "external states", along with their probabilistic dependencies and the prior knowledge (e.g., a previous, correct target morphology). The second step (B) requires specifying mathematically the exchanges (intercellular signalling) between the cells. Because the approach assumes that, for each cell, the behavior of (some or all) the other cells constitutes the "external state",

specifying the interactions between cells corresponds to specifying how the "active states" of one cell changes the "sensory states" or (some or all) the other cells. Intuitively, the active state of one cell might correspond to emitting chemical and/or physiological signals which can be sensed by other cells (the model requires specifying for example the gradients and concentrations that underlie this sort of intercellular signaling. The third and final step (C) corresponds to simulating the dynamics of the problem to find the solution that minimizes the free energy of the (collective) system. A MATLAB toolbox implementing a variational message passing scheme for free energy minimization is the SPM academic freeware (http://www.fil.ion.ucl.ac.uk/spm/); see also [81]. Images reused according to the Creative Commons license from references [85, 160].

Figure 7: Cognitive neuroscience paradigms and their application to models of pattern formation

(A) The TOTE model of a cybernetic goal-directed process. Figure adapted from [174]. Words in *Italics* represent the main processes composing the principle. Thin arrows represent information flows. The double-headed arrow represents a process of comparison between the *desired* and the *actual* state value. The process starts from a Test. If the Test fails (i.e. a mismatch is detected between desired and actual state) an action is triggered (dashed arrow) that causes a cascade of effects such as a change in the actual state that are sensed and used in the next Tests. When the Test succeeds, the process ends. (B) The same model applied to a regenerative context, in which comparison of current anatomical state to a stored target morphology generates signals for cell growth, differentiation, and movement that progressively restore pattern. Cognitive neuroscience is also an example of a field in which high-level information has causal power and is mechanistically integrated with low-level (molecular) details of its encoding and manipulation. (C) Changes of mental state (learning specific patterns for example) alters cell behaviour in the brain (taken with permission from [124b]). (D) Manipulation of bioelectric states in the brain using optogenetic tools is able to insert specific cognitive content (false memories) [78a]. Credit: Collective Next. (E) Conversely, mental imagery can be read out by appropriate decoding of bioelectric state information from living brains (taken with permission from [125a]). In complement to today's models (formulated entirely bottom-up, in terms of molecular pathways), we suggest that successful top-down models of regeneration (in which organ-level topological pattern is represented within somatic cells and guides cell behavior) could be formulated by borrowing insights from cognitive neuroscience.

Figure 8: Applying free energy models to understanding cognition, a "primordial soup", and dynamic morphogenesis.

(**A**) A dynamical exchange between an agent and its environment as modeled in the active

inference framework [81]. Here, a discrepancy between the current sensory state and a goal state encoded in the internal state (reflecting some desired event or the homeostatic level of some variable) gives rise to interoceptive, proprioceptive, and exteroceptive prediction errors (red arrows). This produces a cascade of processes that ultimately enacts a sequence of actions (say, grasping and eating an apple). This process ceases when the interoceptive, proprioceptive, and/or exteroceptive feedback (e.g., the right gustatory sensations) matches the descending predictions (blue arrows) meaning that the organism has restored homeostasis through action. (**B**) A simulation of a "primordial soup" and the emergence of self-organization that is coherent with principles of active Bayesian inference; example from [160]. Left part: This "soup" comprises an ensemble of dynamical subsystems (the dots) that represent macromolecules. The macromolecules have a physical state (representing e.g. their position) and an electrochemical state (representing e.g. concentrations) that change according to simplified Newtonian dynamics and electrochemical dynamics (modeled in [160] using a Lorenz attractor). Crucially, the states have short-range interactions: they are coupled within and between the subsystems comprising an ensemble. Center part: as the system evolves over time, a structure self-organizes that separates subsystems that are conditionally dependent (called internal states) and independent (called external states). Formally, this structure is called a Markov blanket: a kind of "statistical boundary" (more formally the set of node's parents, children, and its children's other parents in a Bayesian network). Note the clear separations - after evolution - in the location of subsystems (macromolecules) with internal states (blue), their Markov blanket (magenta and red), and external or hidden states (azure). States in the Markov blanket can be further subdivided into two sets: those that depend on internal states (red) and those that do not (magenta), called active states and sensory states, respectively. As noticed in [160] in this spatial configuration "the active subsystems support the sensory subsystems that are exposed to hidden environmental states. This is reminiscent of a biological cell with a cytoskeleton that supports some sensory epithelia or receptors within its membrane." Importantly, active states change external states (but are not affected by them) and so they maintain the structural and functional integrity of the Markov blanket. Indeed, "lesioning" internal, sensory or active states (by decoupling them from the rest of the system) quickly leads to the disruption of the Markov blanket - not shown here, but see [160]. Right part: These arguments suggest that a formal analogy can be established between active and sensory states and action and perception systems in living organisms, respectively. This speaks to an even more general interpretation of the self-organization process (shown in the Center part) in Bayesian terms, where the internal states are Bayesian models that infer/represent the hidden (azure) causes of sensory (magenta) states and cause these states through action (red). This can be verified if one considers that sensory states permit predicting external / hidden states - as shown in [160]. (**C**) The same scheme can be applied now to regeneration, where the "internal" (biochemical) states essentially encode a target morphology that can be acquired through a learning process that obeys to free energy minimization processes (e.g., as shown in B) or using unsupervised learning in generative architectures as explained

in the main text. Once the target morphology is acquired, the same error-correction mechanism explained in (A) permit to trigger (regenerative) actions that restore it when it is disrupted. Images reused according to the Creative Commons license from references [85, 160].

Tables:

Table 1: conceptual mapping between cognition and pattern formation

Cognitive concept	Patterning concept
Action potential movement within an axon	Differential patterns of V_{mem} across single cells' surface
Local field potential (EEG)	V_{mem} distribution of cell group
Intrinsic plasticity	Change of ion channel expression based on V_{mem} levels
Synaptic plasticity	Change of cell:cell connectivity via V_{mem}'s regulation of gap junctional connectivity
Activity-dependent transcriptional changes	Bioelectric signals' regulating gene expression during patterning
Neuromodulation	Developmental (pre-nervous) signaling via neurotransmitters such as serotonin moving under control of bioelectrical gradients
Direct transmission	Cell:cell sharing of voltage via nanotubes or gap junctions
Volume transmission	Cell:cell communication via ion levels outside the membrane or voltage-dependent neurotransmitter release
Synaptic Vesicles	Exosomes
Sensitization	Cells become sensitized to BMP antagonists to stabilize neurogenesis
Functional lateralization	Left-right asymmetry of body organs
Taste and olfactory perception	Morphogenetic signaling by diffusible biochemical ligands
Activity-dependent modification of CNS	Control of anatomy by bioelectric signaling within those same cells
Critical plasticity periods	Competency windows for developmental induction events
Autonomic reflexes	Wound healing
Voluntary movement	Remodeling, regeneration, metamorphosis
Memory	Shorter term: Regeneration of specific body organs. Longer term: Morphological homeostasis over decades as individual cells senesce; altering basic body anatomy in planaria by direct manipulation of bioelectric circuit
Pattern completion ability of neural networks (e.g.,	Regeneration of missing parts in partial fragments (e.g., planaria)

attractor nets)	
Forgetting	Cancer, loss of regenerative ability
Addiction	Limb becomes unable to regenerate without nerve once exposed to nerve
Encoding	Representation of patterning goal states by bioelectric properties of tissue
Visual system feature detection	Organ-level decision making during morphogenesis
Holographic (distributed) storage	Any small piece of a planarian remembers the correct pattern (even if it has been re-written)
Instinct	Hardwired patterning programs (mosaic development)
Behavioral plasticity	Regulative developmental programs and regenerative capacity
Self-modeling	Surveillance of anatomical state by brain
Goal-seeking	Embryogenesis and regeneration work towards a specific target configuration despite perturbations
Sub-goaling in problem solving tasks	Developmental modularity
Adaptivity and Intelligence	Morphological rearrangements carry out novel, not hardwired, movements to reach the same anatomical configuration despite unpredictable initial starting state
Tabula rasa	Cells could be a (semi) universal constructor, able to build any shape that can be specified via the pattern memory code
Age-dependent cognitive decline	Age-dependent loss of regenerative ability
Optogenetic insertion of false memories	Optogenetic induction of regeneration or ectopic organs
Reading of semantic content from brain scans	Detecting differences in target morphology from fluorescent voltage dye data

Legend: possible mapping of concepts in cognitive neuroscience to examples in pattern formation (listed in rough order of level of organization, from low to high descending).

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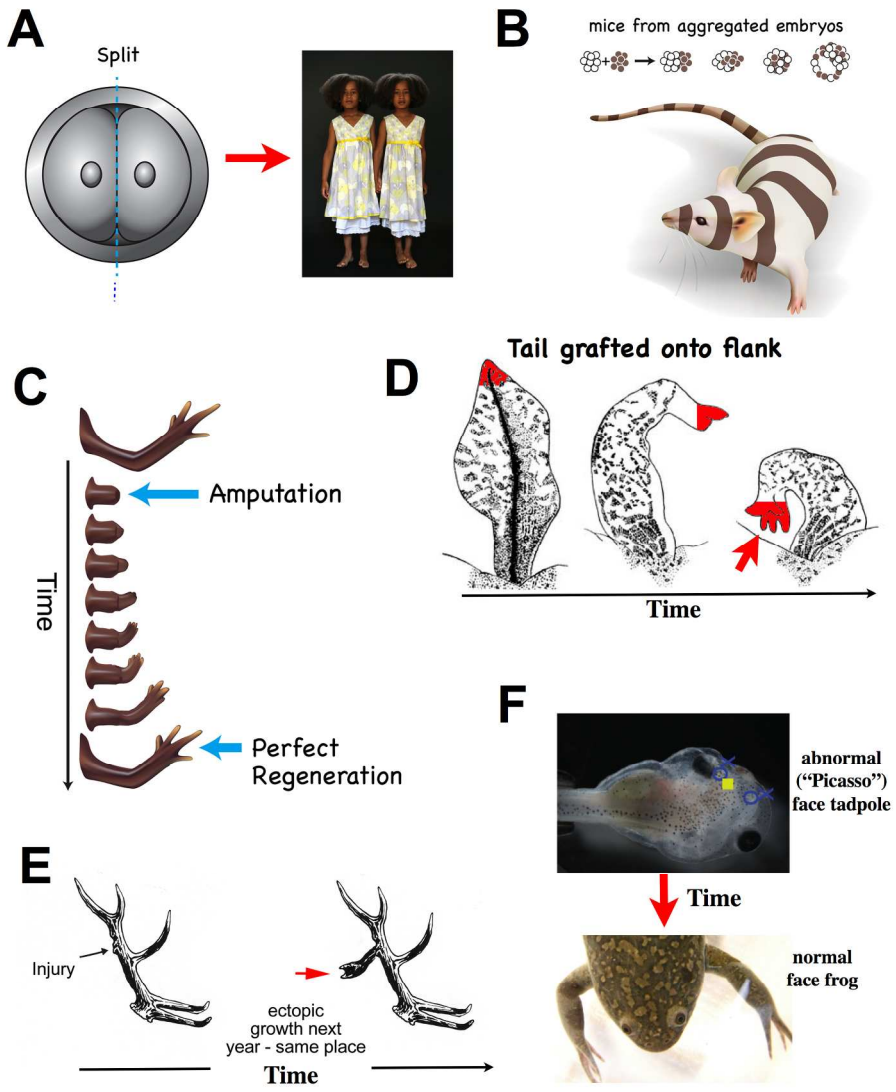

Figure 1

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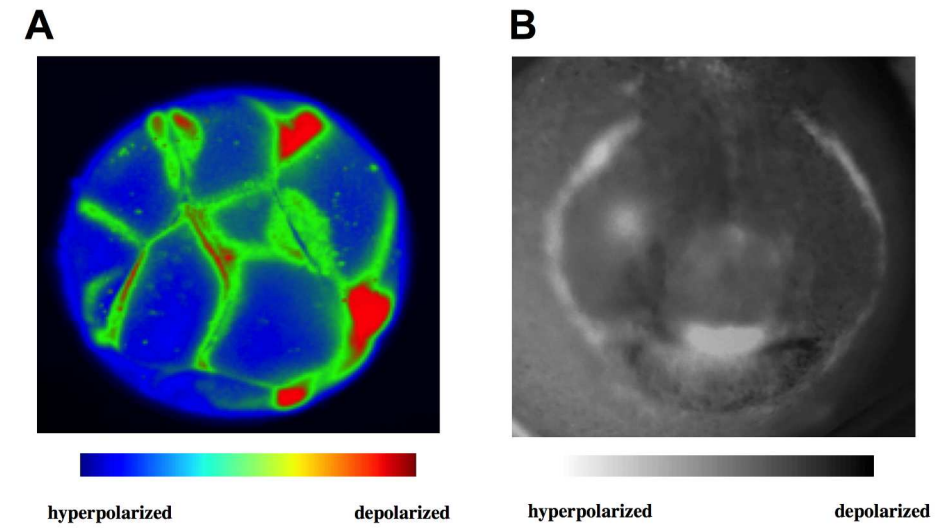

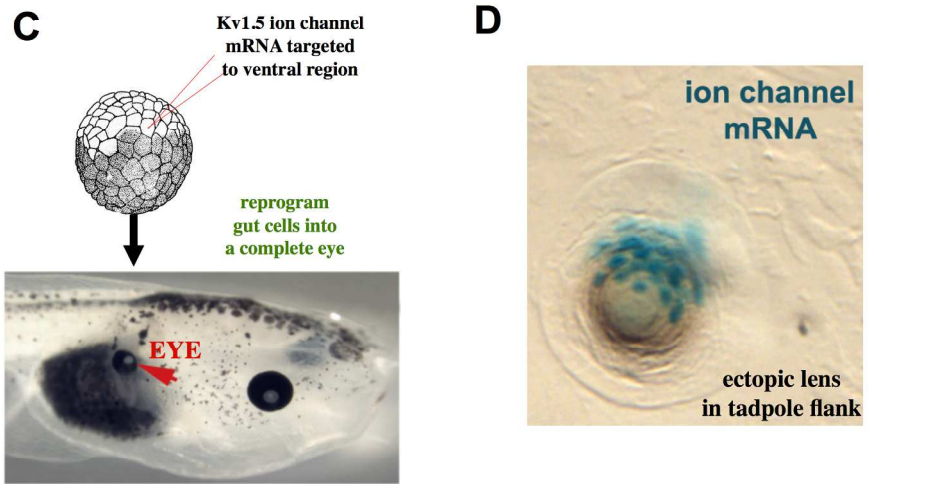

Figure 2

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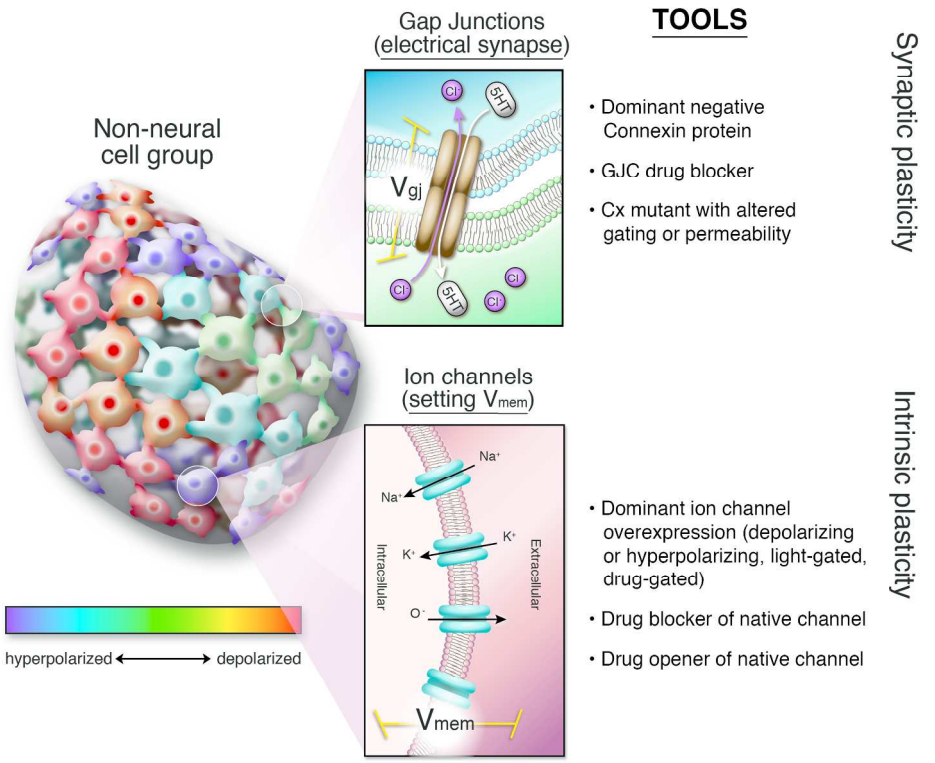

Figure 3

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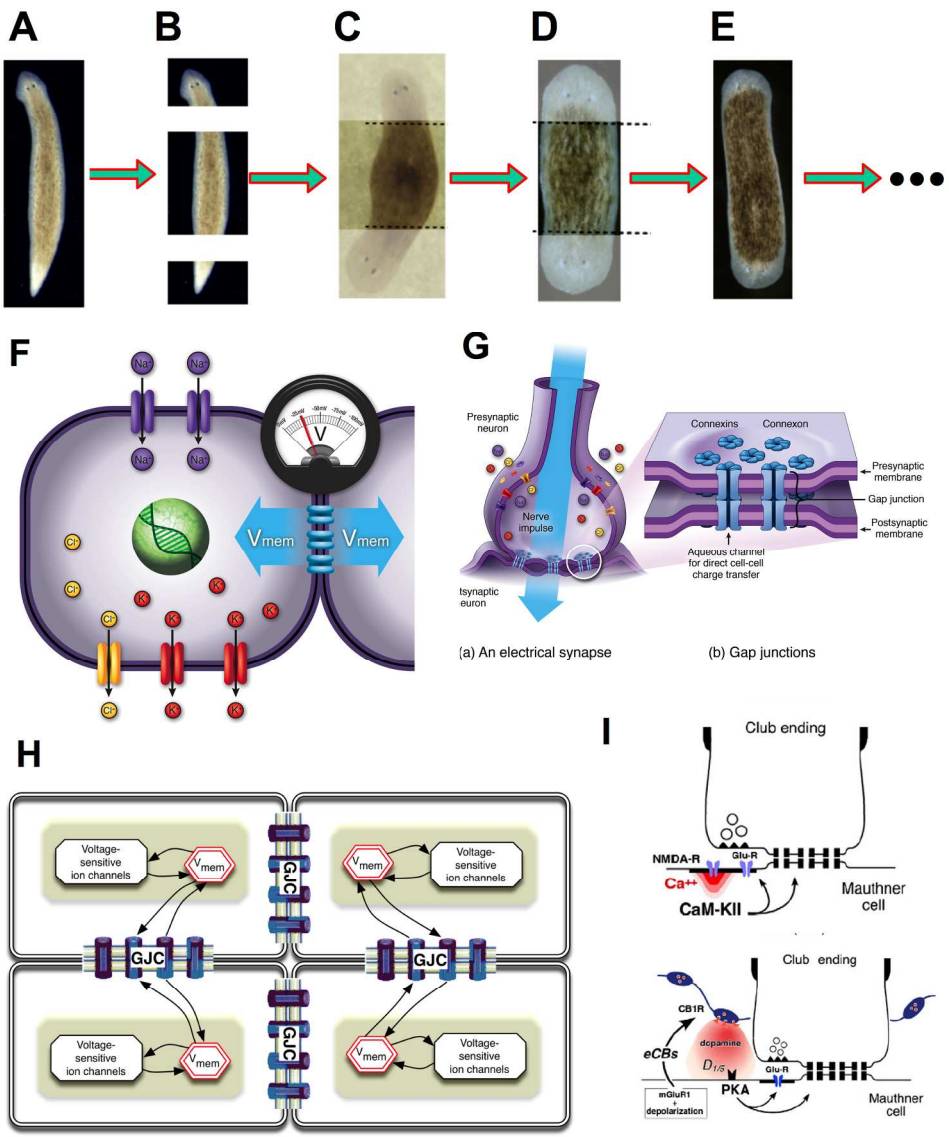

Figure 4

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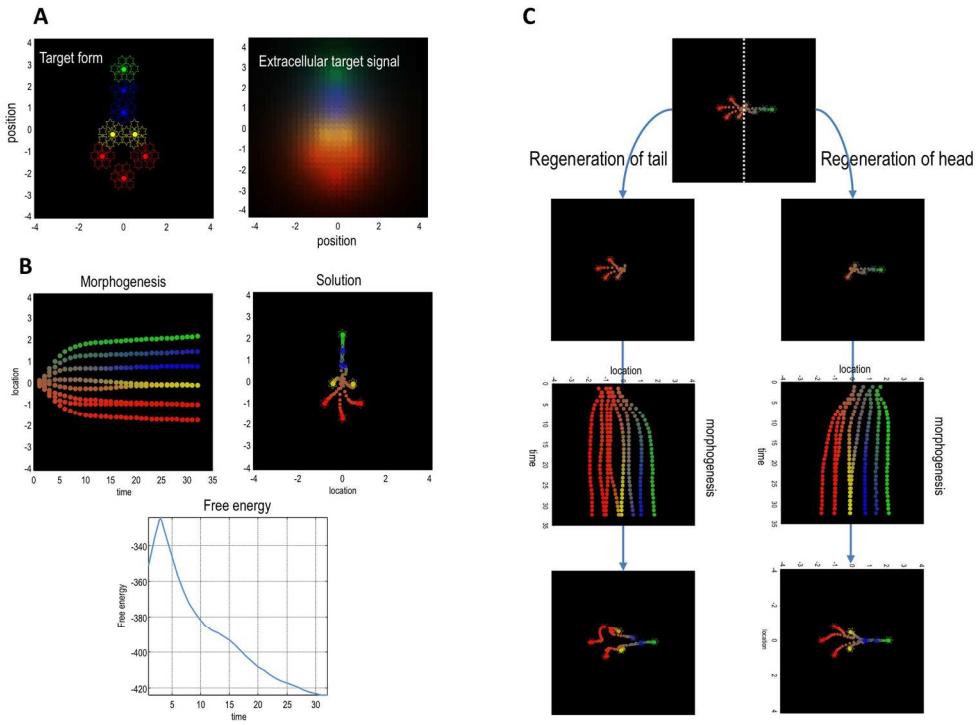

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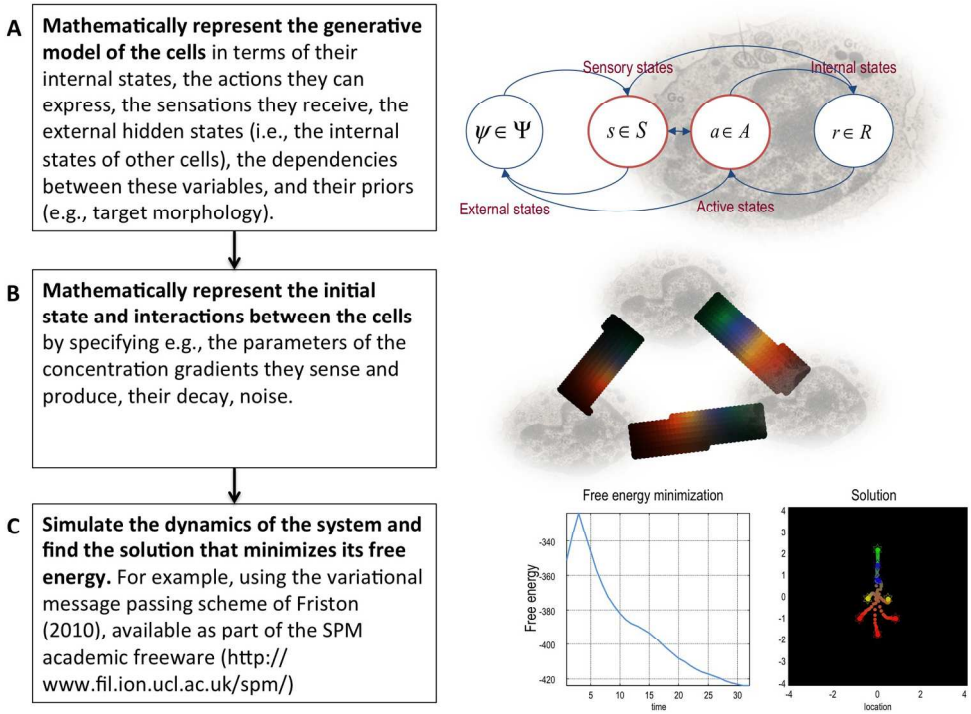

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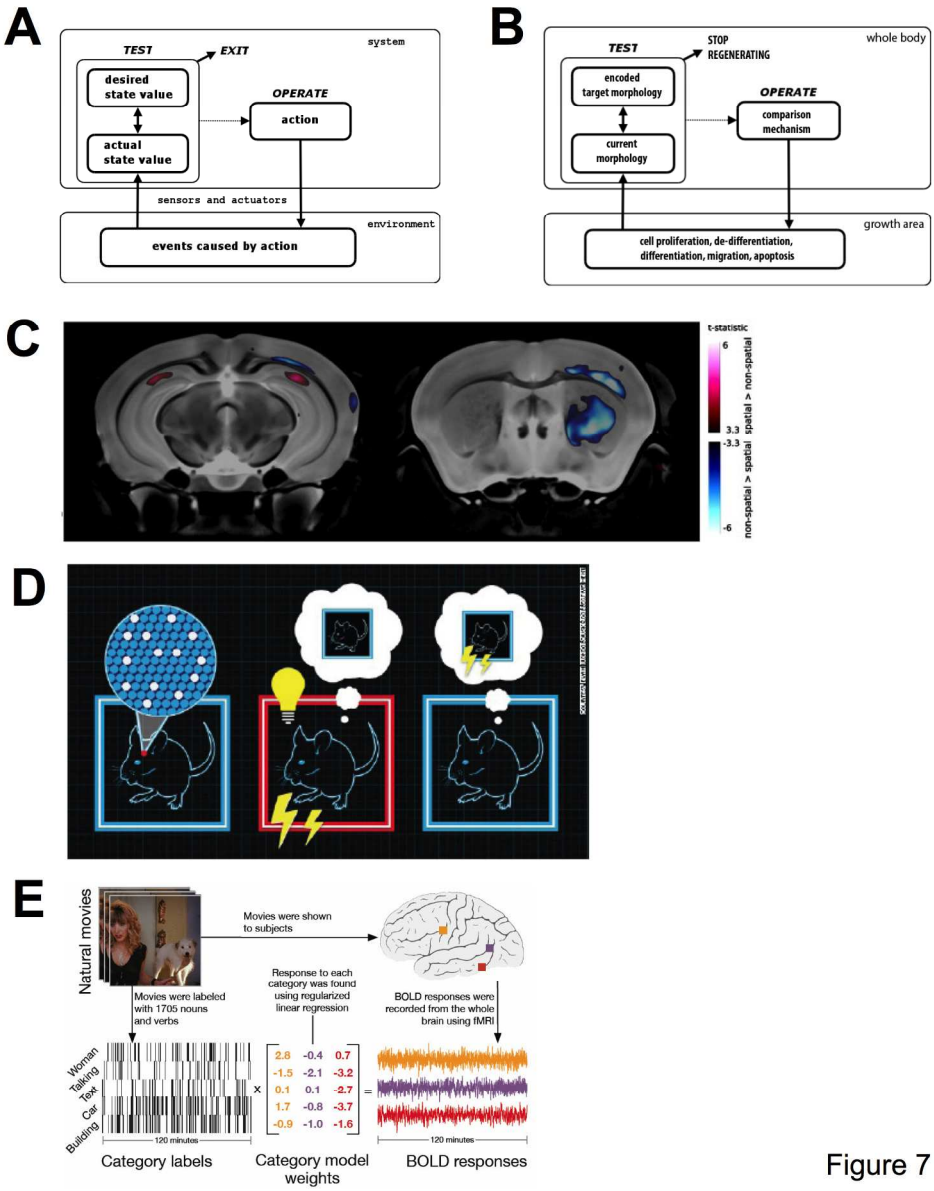

Figure 7

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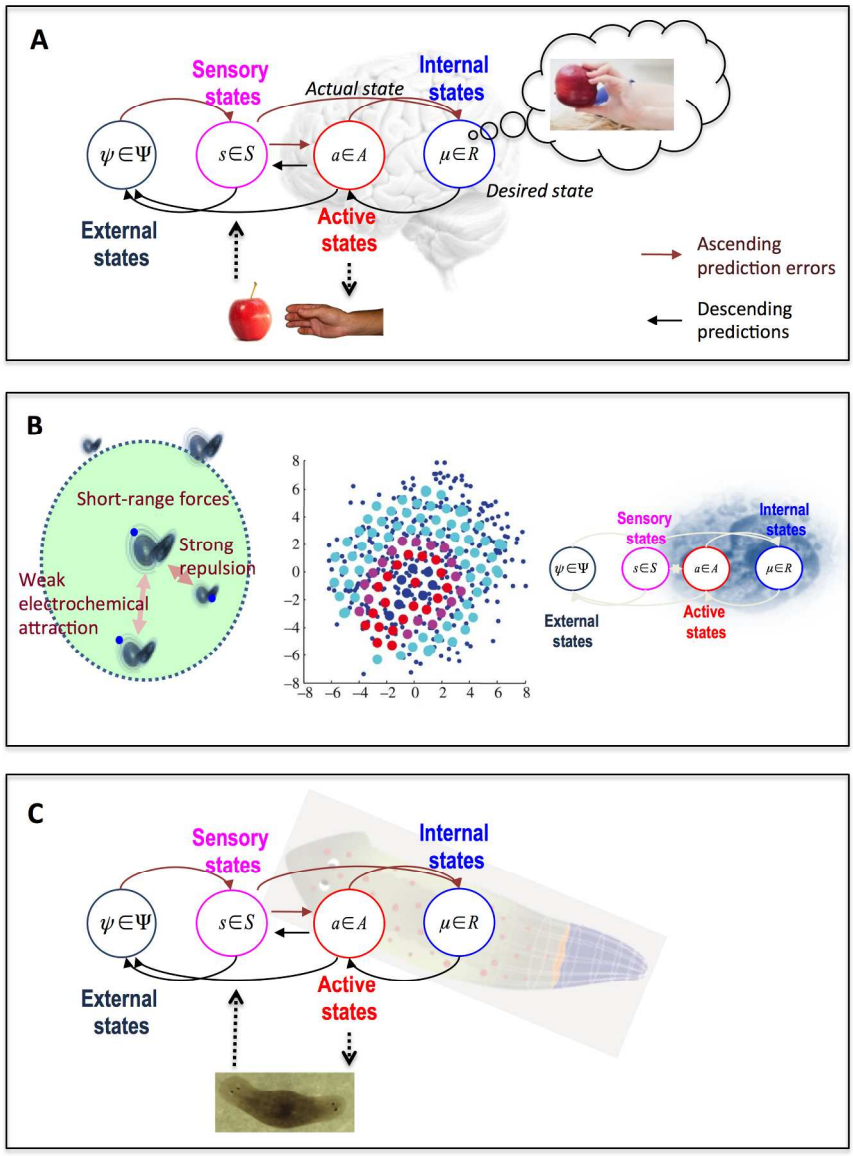

Figure 8

203x253mm (300 x 300 DPI)