Unifying pattern encoding and regenerative remodeling in a connectionist cellular network

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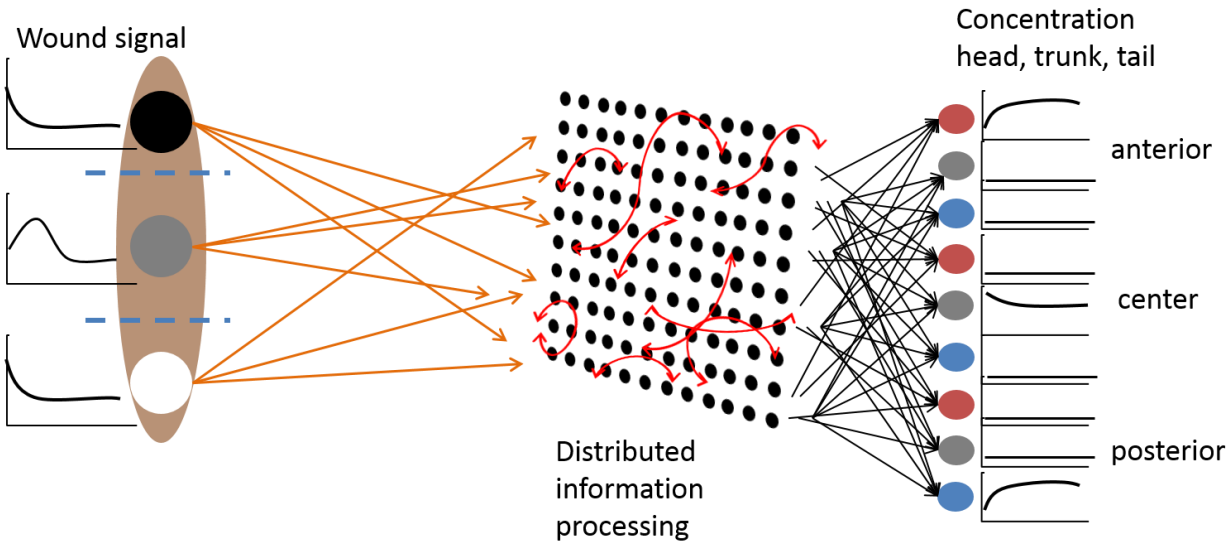

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2 ABSTRACT

Regeneration is a complex process where an organism must programmatically 1) determine what structures are missing and 2) instruct cell proliferation, differentiation, and migration toward the development of complex structures. In contrast to simple wound healing, regeneration of an organ such as a limb is a difficult inverse problem [1]. Bioelectrical communication between non-neural cells is known to have significant control over the development of complex structures, such as inducing ectopic eye development in frog embryogenesis [2]. We propose that the bioelectrical networks that have demonstrated control of patterning in non-neural cells may be better understood through novel models developing from mathematical models of cognition, in particular artificial neural networks (ANN) and reservoir computing methods. In this body of work, we formalize a reservoir computing model of planarian regeneration using positional wound as ANN input and positional tissue information as output and discover that stochastic noise and Hebbian rewiring during training is important for generalization of the response and robustness to perturbation and recapitulate *de novo* experimental results from planarian regeneration experiments. In closing, we suggest future hypotheses to be tested using this model that can be validated at the bench to improve our understanding of the bioelectrical control of patterning during planarian regeneration.

3 INTRODUCTION

Though our understanding of development and regeneration have greatly benefitted from advances in molecular genetics and technologies, we have yet to understand how animals like planaria have the capacity to drive total and perfect regeneration, including from small fragments, up to 1/276th of their body [3]. Planaria are complex metazoans with a CNS and brain [4-6] and are in fact a model organism complete with genetic and molecular tools for studying regeneration [7-9]. In addition to regeneration, planaria constantly modulate their body size to account for energy resources [10-13]. This morphological plasticity is crucial to our understanding of the emergent properties of cell systems, and the important communication between cells that takes place during development and regeneration. As such, planaria are an incredible model for us to better understand the kinds of computation cells undergo to form 3-dimensional structures as well as in tissue differentiation.

Computation is an important concept in this paradigm: upon amputation, the remaining cells in planaria have the capacity to both recognize which structures are lost and go through a series of steps including programmed cell death at the site of the wound [14], followed by proliferation [15], and cell movement [16] to recover the lost structures. Evidence suggests that bioelectrical communication is a crucial driver in development and regeneration of large-scale morphological patterning [17-24]. As the primary mechanism for information processing and higher-order cognition in the brain, individual neurons act within a greater computational system via bioelectrical communication between cells through electrical and chemical synapses (Figure 1A)

acts to form circuits for complex behaviors (Figure 1A'). In non-neural cells, the same kind of bioelectrical communication is possible through gap junctions and voltage-gated ion channels (Figure 1B), which create patterns, like the pre-patterning hyperpolarization in locations that develop to higher-order craniofacial features (Figure 1B') in *Xenopus laevis* embryos [24]. The perturbation of bioelectrical communication can cause functional changes - when the head and tail of a *D. japonica* flatworm are amputated and the trunk is exposed to a gap junction blocker (prevents bioelectrical and small molecule exchange between cells), the amputated piece will regenerate but as a double headed worm [25]. Upon further amputation of the heads in plain water, the planaria will again regenerate a double-headed worm, suggesting a permanent morphology that is not genetically encoded. This field desperately needs computational models to explain and drive further experimental hypotheses that explain how bioelectricity could be acting to 1) detect missing components, 2) instruct morphological recovery, and 3) know when to stop regeneration. The brain also uses bioelectrical signaling to generate higher-order responses. As such, we turn to mathematical models of cognition, developed in the field of computational neuroscience [26], which utilize mathematical models of synapses (Figure 1C) that form an artificial neural network capable of pattern recognition (Figure 1C') and other complex machine learning tasks. Our model serves as the first formal connection between the higher-level organization of classification and decision-making in the brain and the pattern encoding and remodeling during development and regeneration in planaria.

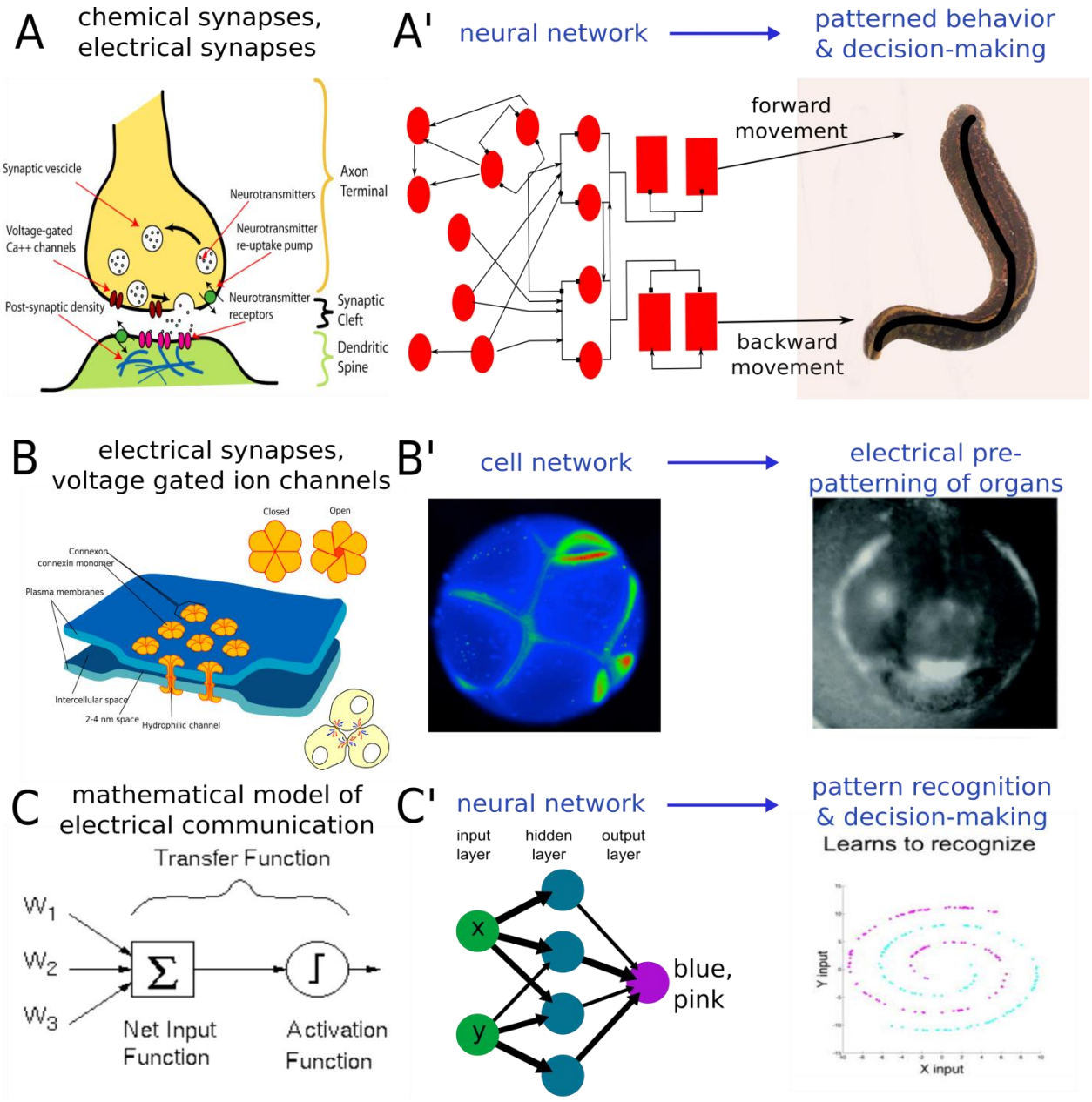

FIGURE 1. Relationship between bioelectrical communication and circuit-based behavior in biological neural networks, non-neural bioelectrical networks, and artificial neural networks. A) Individual neurons in biological networks communicate with other neurons via electrical and chemical synapses (image courtesy of author Nrets distributed through creative commons license), where electrical synapses (gap junctions) quickly conduct electrical signals as well as some small molecules, including iP3 and Ca^{2+}, and chemical synapses communicate indirectly through neurotransmitters that open ion channels as well as other long-term changes. A') Neurons together build computational circuits that lead to complex high-level behavior like swimming [27] in leech (photo courtesy of Karl Ragnar Gjertsen distributed through creative commons license). B) Non-neuronal cells also possess membrane potential, which can be

modulated by gap junctions (image courtesy of Mariana Ruiz, Wikimedia Commons) and voltage-gated ion channels. B') In developing Xenopus embryo, differential membrane voltage in the cells self-organizes and is an eventual marker for pre-positioning of eyes and mouth, predictive of higher-order tissue specification (image from [24]). C) Artificial neurons are connected to other neurons by weight values, and summed with an activation function, typically sigmoidal (all-or-none) and C') when formed into neural networks, learn to classify complex patterns, by x,y coordinates distinguish between two-spirals (image with permission from [28]).

3.1 BIOELECTRICITY AND MOLECULAR CONTROLS OF 3-DIMENSIONAL PATTERN FORMATION IN PLANARIAN REGENERATION

3.1.1 Anatomical pattern detection

Despite great advances in our understanding of planarian regeneration, it is still unknown exactly what physiological mechanisms are in place for a partial worm to faithfully 1) identify missing structures and 2) cease once these structures are recovered to create a fully regenerated organism. Distinct from simple wound healing, planaria upon amputation are able to regenerate complex structures. Upon amputation, neoblasts (adult stem cells) begin to undergo mitosis [29] followed by migration and a second round of mitosis at the site of the wound. Recent evidence suggests that both a generic wound response (produced in all cell types) as well as a cell-specific wound response (produced in stem cells, muscle, or epidermis) precedes regeneration, and notum is the only gene differentially expressed between head-facing and tail-facing wounds [30]. Current molecular technologies allow for broader analysis of wound gene expression following amputation of planaria, and perhaps even more notably demonstrate that these genes are conserved across metazoans [30, 31]. Another known crucial component for anatomical pattern detection for tissue regeneration in planaria is the functioning central nervous system [32]. It has been found that the

ventral nerve cord in conjunction with gap junction communication determines whether a head forms at the site of a wound [25]. This suggests that the communication between neural and non-neural cells plays a crucial role in determining which structures to regenerate upon amputation, but the exact mechanism of when and how these two forces act is still primarily unknown.

Once regeneration has been initiated, another equally important event in successful regeneration that suggests intrinsic recognition of target morphology is the ability to stop cell proliferation when amputated structures are full restored. The activation of extracellular signal-related kinase (ERK) as well as mitogen-activated protein kinase (MAPK) signaling are required for neoblasts to stop dividing and begin differentiating, as demonstrated by pharmacological experiments [33]. Addition to regeneration, the ability for planaria to regulate body size according to energy resources [34] is another such example for which perfect control of initiating and ending cell division is necessary; quantitative descriptions [35, 36] and models of this process seek to describe the relationship between cell number and proportion, an important step in modeling the control of body size in planaria [37].

These questions relating to the anatomical pattern detection capabilities of distributed cell systems can be better understood by taking an engineering approach [38, 39]: what is the minimal information that is needed to determine 1) what structures are missing and 2) how to build and connect missing structures to the incomplete worm? Importantly, the answers to these questions would serve us in mammalian systems in addition to our understanding of basic biology as potential mechanisms for new treatments in regenerative biomedicine and cancer.

3.1.2 Regenerative remodeling and morphological plasticity

Even if the means by which a system of distributed components could detect deviations from anatomical patterning, as is required for regeneration, we cannot yet explain how this system can cue complex tissue regeneration and shape. How do the brain, connected central nervous system, and eyes develop as tissue and become positioned correctly from the trunk fragment of a planaria? How do cells know how and when to proliferate, differentiate, and migrate to faithfully recreate the characteristic head shape of *D. japonica*?

These puzzles are further amplified by experiments that test the plasticity of planarian morphology. When the head and tail of an amputated *D. japonica* flatworm is placed in octanol, the worm regrows a head at each end [25]. Upon a second amputation of the heads in plain water, the worm will faithfully regrow both heads, maintaining this novel double-headed morphology. This plasticity not only suggests that there is an intimate connection between the ability to detect missing structures and ability to dependably regenerate them (which is shown by the planaria's ability to fully regenerate heads after amputation), but also that the memory 'correct' morphology of the worm is not necessarily static – it can be modified to alternative body plans. If the target morphology of a worm can be set and reset, the cells within a single planaria must have an innate flexibility to respond to any and all regenerative instructions. The pluripotent capabilities of adult stem cells (neoblasts) within planaria have been well studied. Recent evidence suggest there is a heterogeneous composition of neoblasts that can with different genetic markers [40] indicating that neoblasts are in fact specialized to a specific cell fate [41]. This even further suggests that coordination is a

crucial aspect of regeneration, as cell types must be balanced between these different neoblast populations.

Another such example of the morphological plasticity of planaria during regeneration is the recent research discovery that upon amputation of their heads in octanol, the species *G. dorotocephala* stochastically regenerates heads that match to wildtype *S. mediterranea*, *D. japonica*, or *P. felina* [42]. The probability of regenerating the shape of a given species was proportional to its evolutionary distance from *G. dorotocephala*. Strikingly, the similarities extend beyond head shape to both the brain morphology and distribution of neoblasts. These inter-species changes speak to the great morphological plasticity exhibited by planaria and the potential impacts of bioelectricity on large-scale patterning during regeneration. In particular, these data suggest a concrete role for bioelectricity in controlling exact tissue and shape formation during planaria regeneration.

3.2 EXISTING MODELS OF PLANARIAN REGENERATION

The first model of planarian regeneration was proposed by Morgan, who suggested a gradient from head to tail controlled patterning [43]. Following this were other gradient theories including Child's gradient descent [44, 45], Spemann organizer [46],, and Wolpert's positional information [47]. Gradient theories alone cannot explain how cells very close in proximity from a center crop of the worm, that were close together prior to amputation can cue information for entirely different tissue types. For an in-depth review of models of planarian regeneration, see [39].

Cellular models have also attempted to explain the ability of planaria to regulate body size and neoblast to differentiated cell proportions [37]. Other models have been suggested to describe certain properties of regeneration without reference explicitly to planaria, in particular focusing on the idea of minimization of free energy and memory as mechanisms underlying distributed information processing across cells during regeneration [48-50]. Despite the importance of these models to study the level of complexity at the cellular level, to understand the minimal components necessary for complex dynamics like regeneration, they are not connected to the idea of regeneration as a new kind of cognitive problem.

A gene regulatory model of planarian regeneration has been reverse-engineered from experimental data [51], using genetic programming as heuristic to discover a comprehensive gene regulatory network *de novo.* This provides the first comprehensive explanation of the genetic gradients across the worm that can be used to describe the sequence of events during regeneration, and tie these to morphological outcomes. This model, however, cannot explain how double-headed morphologies persist beyond one generation, when the information is not genetically encoded. As such even comprehensive gradient models cannot explain all experimental results.

Taken together, gradient, cellular, and gene regulatory network models explain some but not all experimental results relating to planarian regeneration and most importantly do not incorporate bioelectrical communication as a crucial component and predicted computational control of large-scale structure formation. We seek to develop the first bioelectrical model of planarian regeneration that links cognition and development, and regeneration together as information processing problems.

3.3 BIOELECTRICAL MODELS OF COGNITION

3.3.1 Traditional artificial neural networks

In examining the connection between pattern recognition and memory, we turn to the brain. In fact, ion channels and gap junctions are much older than brains [52, 53], and were in use before the evolution of bioelectrical control of behavioral mechanisms [54-56]. Artificial neural networks are used for both biological modeling as well as in machine learning and artificial intelligence. These are two distinct areas of study with that suffer from their own unique limitations. Exact mechanistic modeling of the brain is very difficult, though in the advent of modern computing becoming a more feasible task, and artificial neural networks for machine learning are suggested to be too symbolic to represent biological reality, though they possess some characteristic behaviors of biological neural networks.

Traditional artificial neural networks are characterized by the basic functioning of biological neural networks. They are made up of individual cells which are connected to other cells by weights which imply strength of the synaptic connections. The output of a neuron is computed by the summation of the potentials of neighboring neurons, and transformed by a potentiation function. Many artificial neural network structures exist, and the merits of their biological significance and machine learning potential are often discussed. Artificial neural networks can be used to approximate functions [57], classify complex patterns [58], and interpret time series [59]. Different network structures in terms of the functions for neurons and the ability to have recurrent connections are known to determine the limitations [60] of the networks' ability to learn complex behaviors. For example, feedforward networks with simple integrate-and-fire neurons

can be trained to approximate functions, but lack a “memory” of previous inputs. The incorporation of either spiking neurons [61] or recurrent connections [62] gives a network a memory within the cell substrate, and in some networks, including Hopfield networks [63], leads to stable states called basins of attraction [62, 64] that have real biological groundings [65, 66], which once a stable state is reached, it becomes more difficult for subsequent inputs to cause a change in output.

3.3.2 Reservoir computing

Reservoir computing is a recent artificial neural network architecture developed to solve the difficult problem of training large biologically relevant recurrent artificial neural networks [67]. Instead of training the network itself, it trains a detector to recognize patterns within the reservoir and interpret them as output, most commonly a perceptron. A major benefit to reservoir computing is the detector does not need to have any biological relevance, as the information is still contained within the reservoir, but just extracted by some pattern-recognizing program and thus can be selected for simplicity and speed. It is assumed that the reservoir itself has the capacity (memory) to store the temporal input pattern among the nodes, and the detector simply acts as a linear regression layer to obtain the network’s readout [68]. The reservoir is called such because the inputs the network can be thought of as rocks being thrown into the “reservoir” of water and causing surface ripples who patterns can thus be interpreted. Liquid state machines (LSM) by [69] and echo state networks (ESN) [70] are the two major forms of reservoir networks that were developed independently. They are very similar except in choice of neuron integration function [71]. Reservoir computing is highly regarded as closer to the reality of biological neural networks than more

traditional ANN models, yet has tools for optimization of the detector available to process more difficult problems and do not require a loading of basins of attraction as with Hopfield networks or other recurrent networks. This allows for complex dynamics not specified by the user to emerge.

3.4 SHAPE AND TISSUE DETECTION PROBLEMS LEARNED BY ARTIFICIAL NEURAL NETWORKS

3.4.1 Shape

There is an innate connection between symbolic representations and shape: our language system is entirely dependent on the ability to identify differences between shapes, for example a ‘5’ and ‘3’ or the coordinates within a spiral. Another such example is in facial recognition. Humans can identify the difference between face shape in both 1) recognizing individuals as well as 2) recognizing emotion. This also exemplifies an ability to mix identification of distinct patterns with an ability to generalize. The same capacity to cue both general and specific patterns is in place in morphological pattern recognition during the initial phases of regeneration: general and specific wound signals are both required to accurately recover lost tissues [40].

Artificial neural networks are well-established as tools for morphological pattern classification problems. Their flexibility to understand many different kinds of symbolic problems has been well documented. Most recently, deep networks have become a recent gold standard in face recognition [72] and hand-writing recognition tasks [73]. This suggests that the ability to fine-tune *in silico* circuitry mimics the real expertise of the brain.

3.4.2 Tissue detection

Artificial neural networks have been proven capable of classifying cancerous and non-cancerous tissue using genetic information [58, 74] and using image analysis [74, 75], and for detecting other tissue defects [76-79]. As a standard machine learning tool in biomedical research for tissue subtype identification with measurable success, the artificial neural network is already a tool that from low-level features can extract higher-level classes, in either a supervised or unsupervised manner. Evidence suggests that the bioelectrical cell network of planaria must compute long-range information to coordinate regeneration that can be perturbed to develop double-headed worms. To really mimic the proposed non-excitable bioelectrical cell network that controls shape and tissue specification in planaria, a bioelectrical network model would have to not only detect tissue type, but also drive tissue specification. As it stands, this is a novel application of artificial neural networks to be both pattern detectors and pattern generators simultaneously.

3.5 BIOELECTRICAL NETWORK PLASTICITY

3.5.1 Plasticity and the brain – excerpt from Hammelman et al, 2016

It was once a long-standing central dogma of neurobiology that the central nervous system was incapable of accommodating neuron growth and death. This theory has since been overturned [80-82], which opens up a new field of study to understand how individual neurons and their environment contribute to the overall plasticity and growth of the brain [83, 84]. Much evidence now suggests that biological neural networks in many organisms, including mammals, have the capability of pruning,

growing, and altering connectivity [85, 86]. Some species of insects undergo significant remodeling of the central nervous system and brain during metamorphosis from the larval to adult form, yet have been shown to maintain stored memories. Drosophila and manduca larvae have an ability to remember aversive associative learning tasks pairing electric shock with odor into adulthood [87, 88]. Weevils and wasps demonstrate a preference for the odors of their larval environments, supporting a long-standing hypothesis in entomology known as the Hopkins 'host-selection principle' that beetles and other insects favor their larval environment in selection of their adult homes [89, 90]. Planarian flatworms are another animal that demonstrate persistent memory after undergoing extensive remodeling. These flatworms have an incredible regeneration capacity in that they can regrow a fully functioning central nervous system and brain after head amputation [91]. In studies of memory persistence after head regeneration, it was discovered that flatworms trained on learning tasks before amputation require significantly less trials to re-learn the same task (the "savings" paradigm) than amputated but previously untrained animals [92, 93].

While amphibians, zebrafish, and planaria are capable of central nervous system regeneration, it appears that most mammals and birds are more limited in neurogenesis to specific regions of the brain [85]. Human neuronal progenitors have already proven to be a promising candidate as a treatment of traumatic injury, shown to successfully integrate and aid functional recovery in mice [94, 95]. Conversely, elevated neurogenesis in the dentate gyrus region of the hippocampus is a cause of forgetfulness in adulthood and infancy [96]. Neuronal death is also implicated as a primary cause of neurodegenerative diseases, especially diseases like amyotrophic

lateral sclerosis, where motor neurons weaken and die slowly over time, and in traumatic brain injury, where delayed neuron death occurs in selective cell regions [97]. Yet cells also die during initial neurogenesis in the development of the nervous system as a pruning mechanism for neurons that are poorly wired or functionally redundant [98]. Understanding and leveraging the neural plasticity of animals capable of brain repair is the first step for finding a treatment of traumatic brain injury: replacing or inducing formation of lost neurons and allowing the network to dictate their differentiation and functional response, which will contribute to innovative solutions for treatment of traumatic brain injury and neurological disorders [99].

Despite the rapid move toward stem cell therapy for degenerative disease and brain damage, it is still completely unknown what the cognitive consequences will be for an adult patient with decades of memories when the brain is engrafted with descendants of naïve stem cells. The studies of morphogenetic remodeling mechanisms and of memory and behavioral performance have not been integrated. Indeed, there are no established platforms for computational modeling of behavior during brain remodeling. It is thus imperative to begin to formalize the understanding of what happens to memories and behavioral programs when cells are added (proliferation), removed (apoptosis/necrosis), relocated (migration), and re-wired (synaptic plasticity).

3.5.2 ANN simulated perturbation for optimization

Currently, the machine learning capabilities of artificial neural networks come at the cost of transparency: it is generally accepted that artificial neural networks are a “black box”, though there have been many attempts to extract rules from the complex

internal dynamics of the network [100-103]. Given the incredible capabilities of these models as pattern recognizers, it is a crucial line of research to develop state-of-the-art methods to understand artificial neural network function. One way to better understand these networks is to perturb them similar to as we would in biological experimentation. The discovery of adult neurogenesis in the hippocampus [83] has become a motivating factor for computational neuroscientists to incorporate neuroplasticity into neural network models [104].

3.5.2.1 Optimization of ANN Structure Excerpt from Hammelman et al

While most of the work to-date has focused on ANNs with constant (fixed) topologies, a few studies have exampled dynamical changes to ANN structure. Artificial neural network growth and pruning techniques have been primarily addressed as a part of training to avoid over-fitting (network is too large) or under-fitting (network is too small) [105]. The majority of techniques developed are meant as a pre-training network optimization or an intermediate step in training the network and therefore the immediate effects of perturbation on memory persistence after all training has been completed have not been studied [106-108]. While some studies of artificial neural network architecture have attempted to draw connections between such computational methods and their biological implications [105, 108], none have formally attempted to test perturbation methods prior to re-training the network.

The combination of genetic algorithms and artificial neural networks has given rise to a series of studies related to evolving adaptive and developmental neural networks [109-113]. These and other works studying neural-like cellular models of development [48, 114] suggest that artificial evolution has the capacity to produce

robust information processing systems. Compositional pattern producing networks (CPPNs) are an example of a developmental artificial neural network model evolved by genetic algorithms [110, 112], and incorporate protein diffusion along with excitatory proteins into an artificial tissue model. This model was later extended with the neuroevolution of augmented topologies algorithm, NEAT, [115] which provides a method for augmenting artificial neural network topologies to become incrementally complex throughout genetic algorithm evolution [109]. Most recently, the CPPN-NEAT method was used to evolve 3-dimensional structures, a promising avenue for the study of morphogenesis [113].

ANN architectures developed in the modern age of neuroscience must address neuroplasticity as a method for improving training performance as well as reflect the biological reality. Of note are two such ANN models that have been developed in the last few months, which are designed to incorporate neurogenesis and neural cell death into the system itself. DRASiW, a weightless neural system, uses special RAM nodes that store information about unseen patterns, and with DRASiW the prototypes of learned patterns as well [116]. The authors provide an algorithm for changing the bit resolution for memory addressing by join (apoptosis) and cut (neurogenesis) operations and prove it either minimally degrades memory in the case of join operations or perfectly maintains memory in the case of cut operations. The other, Dynamic Plastic Continuous Neural Networks, uses a plastic medium “plate” that can be changed during training, and has adaptive connection weights [117] . The recent publications of these two ANNs demonstrate that the development of computational models that incorporate neuroplasticity is currently an open field of study.

3.5.2.2 Reservoir network structures studied under perturbation

Unique in comparison to other ANN structures, there are a significant number of studies addressing the perturbation of reservoir computing networks. In fact, the liquid state machine introduced by [69] included in this original study the effects of adding neurons to the reservoir, which increased the separation of the liquid circuit. Though typically the reservoir is static throughout training, neuronal plasticity between the weights of output and reservoir nodes through learning rules for rewiring has been shown to improve echo state network performance for classification and regression [118] and time series prediction [119, 120] and for a separate self-organizing recurrent neural network structure that incorporated multiple forms of plasticity with specific dynamics for inhibitory and excitatory neurons [121].

Other work has studied the impact of perturbation to the reservoir on the performance of an ANN, and suggested that a small-world network that followed the power law provided the best topology for robustness to synaptic perturbation and noise in liquid state machines [122] and is consistent with known information about the topological connectivity of the brain [123]. This work uniquely studied perturbation *without* subsequent retraining, suggesting the ANN structure is more amenable to perturbation than earlier methods.

3.6 CONTRIBUTIONS OF THIS PAPER

In this paper we will:

- Examine the anatomical pattern detection capabilities of feed-forward artificial neural networks on planarian patterns of shape to develop hypotheses for the computation of morphological information (Appendix)
- Formalize a model of planarian bioelectrical information processing from reservoir computing
- Analyze network characteristics that improves its ability to generalize the response to deviations from input pattern and be robust to perturbation of reservoir node synapses
- Demonstrate our reservoir model mimics real planarian results under perturbation and retraining

4 RESULTS

4.1 A RESERVOIR MODEL OF REGENERATION

We introduce a reservoir model of planarian regeneration, using an echo state network with neuroplasticity in the reservoir using Oja rewiring dynamics (Equation 1). An amputation of a planaria is effectively the input to the worm to signal regeneration (Figure 2A), which we represent as input into our reservoir computing model as three inputs for a specific time point: anterior, center, posterior wound signal (Figure 2A') which we will refer to as "positional wound input". The planaria system is supposed to contain a bioelectric memory via membrane potential (Vmem) gradients and communication between gap junctions (Figure 2B). In our model, this is represented by the reservoir which is capable of remembering time through its recurrent connections

between internal units (Figure 2B'), and in our case like the planaria cell network is self-organizing through Hebbian rewiring. The programmatic regeneration of the network is thought to be a consequence of the bioelectrical memory and through evolution has become effective at regenerating complex organs (Figure 2C). In the model, the output weights are the 'detector' which interpret the firing pattern of the reservoir and are trained by supervised learning to the target output (Figure 2C'). In biology, the sequence of wound, anatomical detection and memory of target morphology, leads to blastula formation, differentiation, and the regeneration of the head and tail of the trunk fragment (Figure 2D). In the reservoir computing network, there are 9 outputs: anterior head, trunk, and tail, center head, trunk, and tail, and posterior head, trunk, and tail concentrations which we will refer to as "positional tissue output" that follow a specific pattern based on the wound input signal (Figure 2D'). The detector for the reservoir is a feedforward perceptron, trained by pseudoinverse algorithm.

(EQUATION 1)

$$\boldsymbol{w_{ij}}' = \boldsymbol{w_{ij}} + \boldsymbol{\alpha} * \boldsymbol{\sigma(j,x)} * (\boldsymbol{\sigma(i,x)} - \boldsymbol{\sigma(j,x)} * \boldsymbol{w_{ij}})$$

Where w_{ij} is the weight between some neurons i and j, α is the learning rate, σ is the output function for a given neuron and some input x.

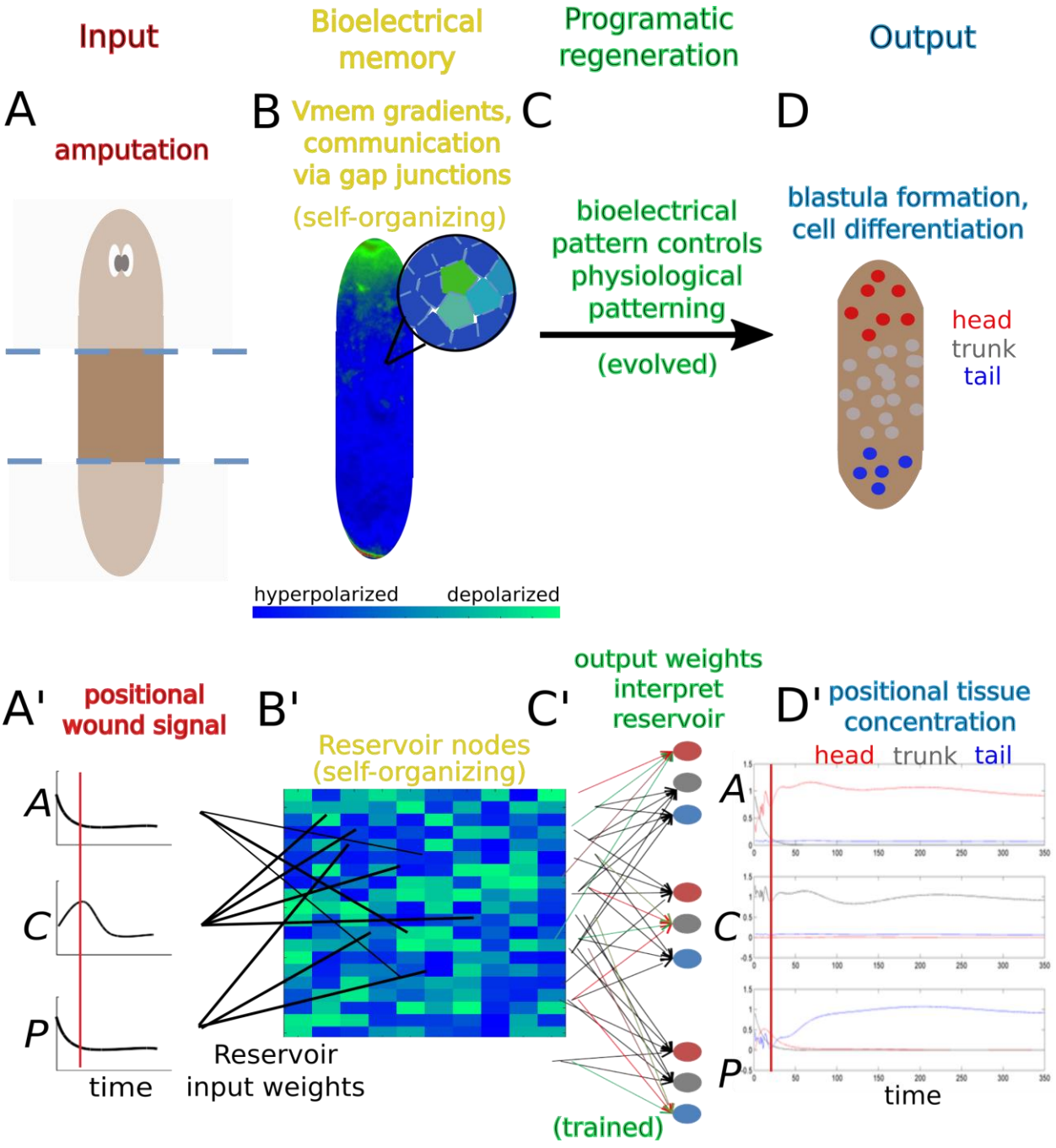

FIGURE 2. Parallels between planaria regeneration and reservoir computing model. A) The head and tail of a planaria, when amputated, causes wounding which signals to the worm to begin regeneration. A') The input to a network is a wound signal at a specific point in time (red line) from the anterior, center, and posterior sections of the worm. B) Membrane potential (Vmem) and gap junctional communication causes a regional gradient, characterized by a depolarized head and relatively hyperpolarized body. B') The reservoir of the network is self-organized as through Hebbian rewiring dynamics, and has cells which are hyperpolarized and depolarized for a given input. C)

The planarian bioelectrical pattern has known effect on physiological outcomes, tuned through evolution. The mechanism for response to bioelectrical signaling is presumed to be transcription of gene regulatory elements that control cell type differentiation to obtain the correct target morphology. C') The output weights of the network are trained through pseudoinverse learning algorithm via supervised learning of the target outputs of positional tissue input and output information. D) The final step in planarian regeneration is the cell proliferation, migration, and differentiation to reformulate the missing head and tail structures. D') The final step is the expected concentrations of head, trunk, and tail and the anterior, center, and posterior sections of the worm.

This network was able to successfully learn the positional tissue output pattern (Figure 2D') in response to positional wound input, with head anterior and tail posterior concentrations rising over time and trunk center concentration remaining static. The anatomy of the reservoir computing model allows for us to study how the planaria regeneration problem is solved by the network, particularly by perturbing the reservoir connections (Figure 3C) and examining the strength of the weights between the reservoir nodes and the output nodes that pattern the positional tissue output (Figure 3D).

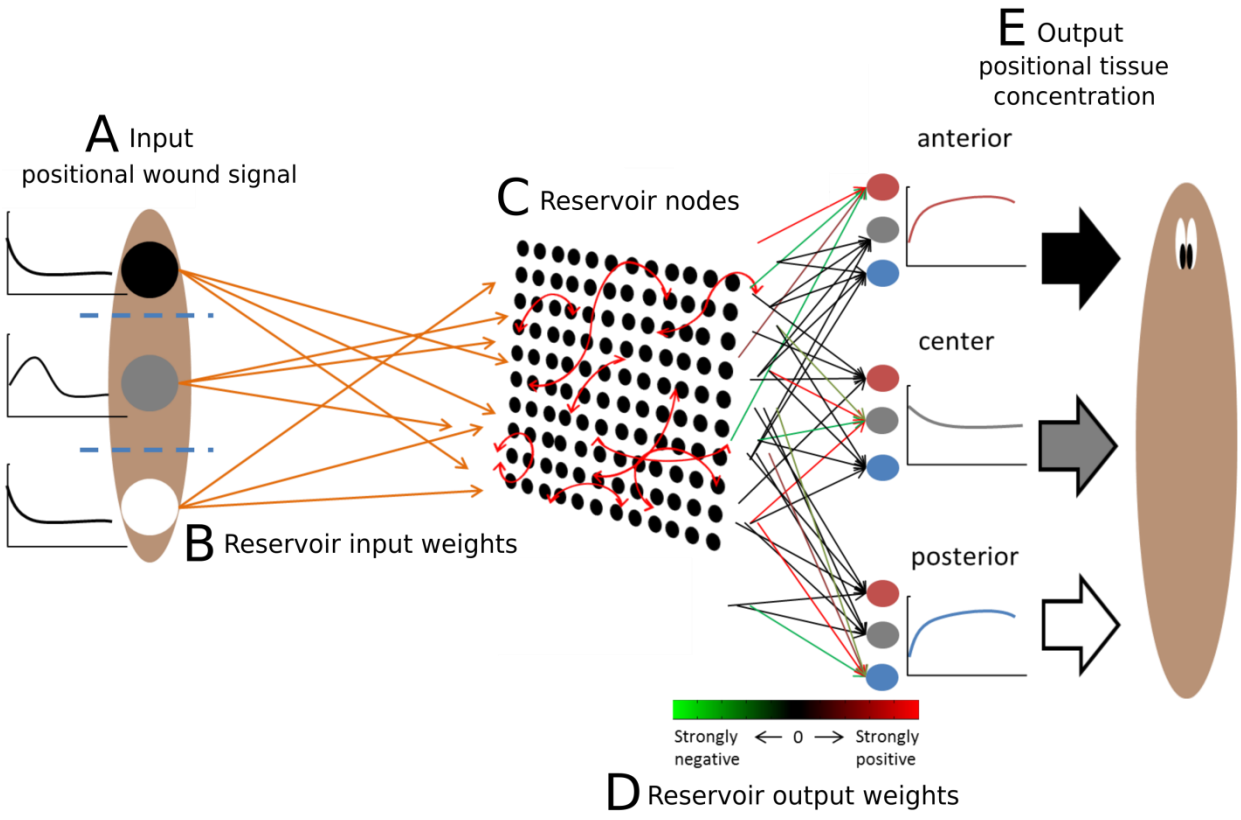

FIGURE 3. Anatomy of a reservoir network model of planarian regeneration. A) Input to the network is a time series wound signal at positions at the top, center, and bottom of the worm that is input to the reservoir nodes. B) The input is randomly connected to the reservoir nodes which process the input. C) The reservoir nodes process the input signal. Reservoir nodes are connected to other reservoir nodes and may also have recurrent connection, and thus are able to compute time-dependent patterns. D) The output weights between the reservoir nodes and the output nodes as the perceptron detector is trained using pseudoinverse to the output pattern by supervised learning to the output pattern. Their strengths tell us when reservoir nodes are heavily contributing to output. E) The output pattern is the concentrations of head, trunk, and tail across the anterior, center, and posterior positons of the worm.

4.2 TRAINING WITH NOISE IMPROVES PERFORMANCE FOR NOISY INPUT AND PERTURBED CONNECTIONS

Using noisy data to train the reservoir increased the performance of the reservoir under novel noise (Figure 4) and perturbation (Figure 5) compared to a reservoir that

was not trained on noisy data. To ensure that this was not just a result of pattern repetition or a unique aspect of one individual topology, we compare the results between 100 ESNs trained on 1) the original input to original output 2) the original input to the original output 100 times 3) 100 noisy inputs (range 0.001 to 0.01) to the original output. Without noise in training, the network did very poorly in generating the expected output pattern on inputs with small levels of noise, suggesting it had not truly learned the underlying network pattern (Figure 4C) and was true even when the input was repeatedly given to account for the rewiring of the reservoir (Figure 4D). When trained with noise, the ability to generate the output pattern under novel noise was significantly improved (Figure 4B). The same pattern was true for perturbation (Figure 5), though all networks performed significantly worse under perturbation than with noisy input.

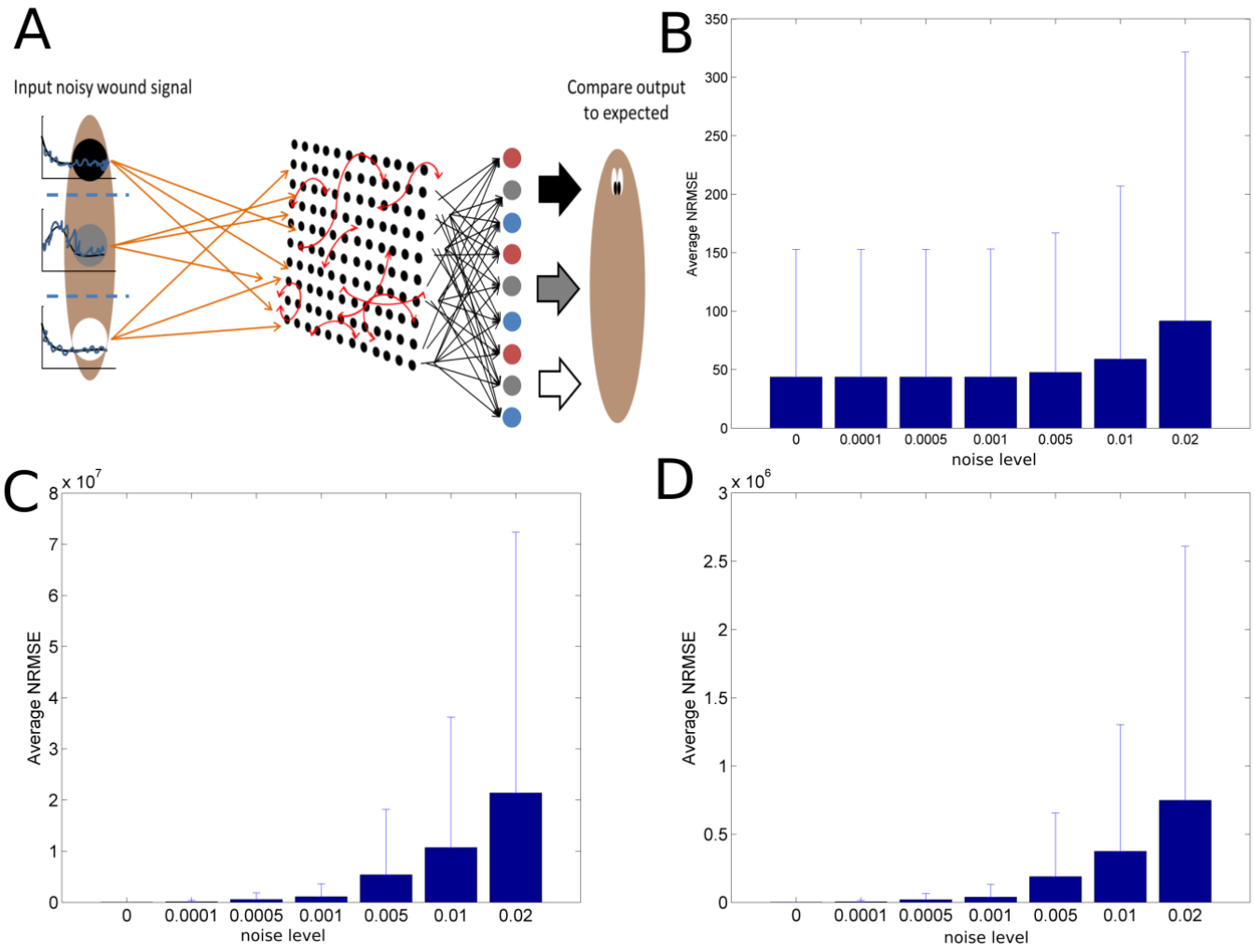

FIGURE 4. Training with random noise improves networks' ability to generalize. A) The network is tested under random noise and compared to the expected output pattern for wildtype positional tissue information under normalized root-mean-square error, this was tested 100 times for each of 0, 0.0001, 0.0005, 0.001, 0.005, 0.01, and 0.02 levels of noise (added relative to average concentration gradient). B) When trained with 100 instances of small random noise, the network is able to regenerate the pattern with equal accuracy with elevated noise levels, suggesting it has learned an underlying relationship between input and output. C) When trained without random noise, the network is unable to recognize the input pattern even under small levels of noise, reaching 10^7 normalized root mean squared error. D) When training is repeated 100 times to account for the rewiring of the reservoir, the network does only slightly better, reaching 10^6 levels of error.

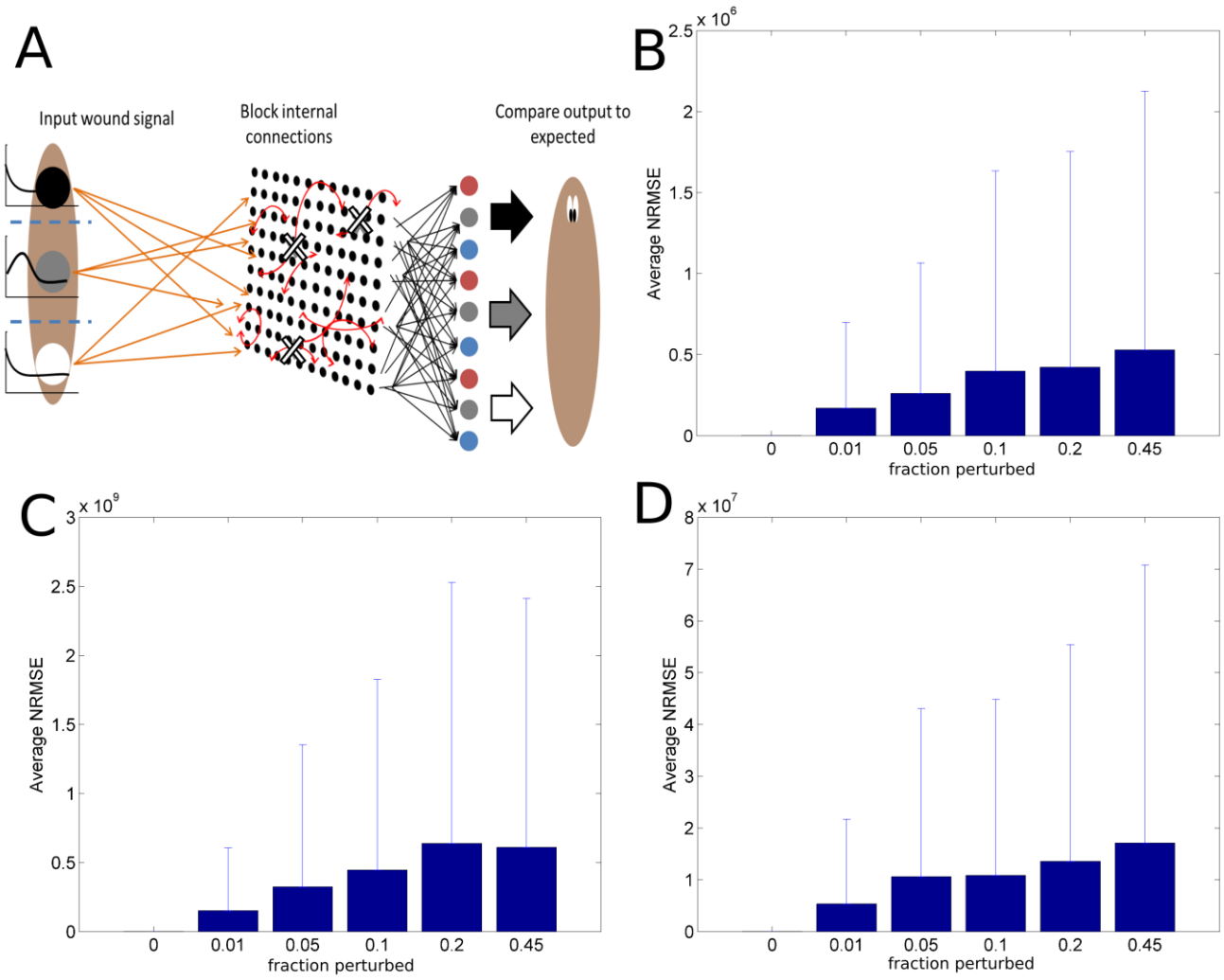

FIGURE 5. Training with random noise improves networks' robustness to connection blocking. A) We train a network and then perturb random connections in the internal reservoir and compare output to the expected wildtype positional tissue information by normalized root-mean-square error. This was tested 100 times for each of 0, 0.01, 0.05, 0.1, 0.2, and 0.45 fraction of the reservoir perturbed. B) Trained on 100 instances of random noise, the network is more robust to perturbation than C) without random noise and D) without random noise and the training repeated 100 times to account for reservoir rewiring.

4.3 TRAINING WITH HEBBIAN REWIRING IMPROVES PERFORMANCE FOR NOISY INPUT AND PERTURBED CONNECTIONS

We trained 100 network instances without the modified Hebbian dynamics and the stochastic noise inputs, and used the same noise and perturbation analysis, and found that the network performed worse under both noise (Figure 6A) and perturbation (Figure 6B) with the noise error being double the results with Hebbian rewiring and with perturbation, there was an order of magnitude difference between the network without rewiring (Figure 5B) and with rewiring.This suggests a significant effect of reservoir self-organizing to improve the performance of the network under imperfect conditions.

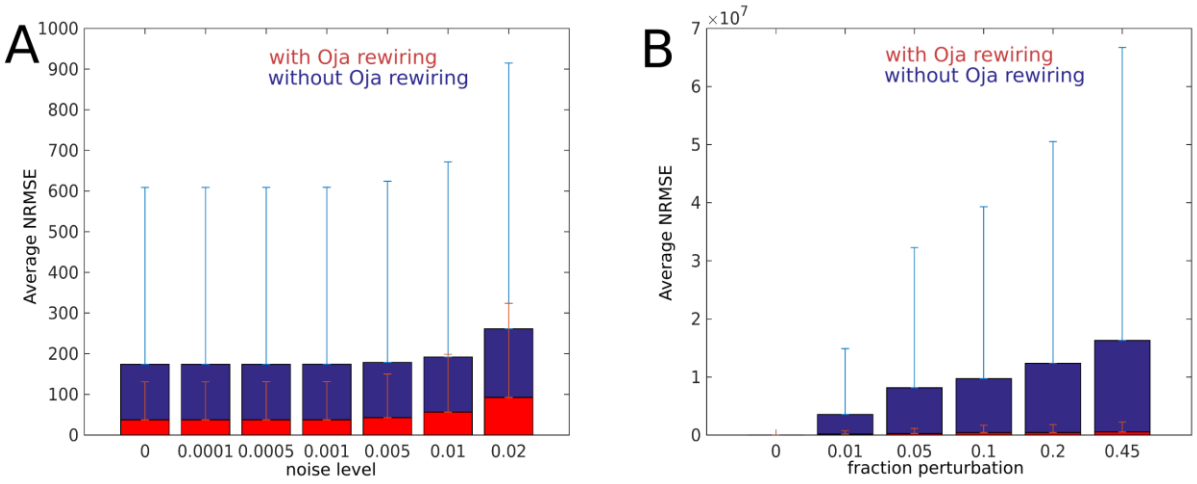

FIGURE 6. Results for noise and perturbation assays without Oja rewiring. A) The normalized root mean squared error values for network trained without rewiring, tested on 100 novel noisy inputs are double the error of the network trained with Oja rewiring. B) The normalized root mean squared error values for the network testing on 100 different perturbations of internal connections are more than 10 times the normalized root mean squared error values of the network trained with Oja rewiring.

4.4 PERTURBATION AND RETRAINING MIMICS PLANARIAN EXPERIMENTAL RESULTS

After training a NN on the planaria regeneration as specified in 4.2, we perturbed the two sides of the reservoir, along with the internal connections as is the supposed impact of amputation and gap-junction blocking experiments that result in double-headed *D. japonica* flatworms (Figure 7A). We then retrained the network on the first 350 time steps of positional wound input to two different output patterns: 1) a double-headed positional tissue output and 2) a wildtype positional tissue output. Surprisingly, the results of the retrained wildtype planaria appeared to have highly active weights in the posterior tail section (Figure 7C), as did the retrained double-head NN (Figure 7B). In planarian trunk fragments treated with octanol, some will regrow to the wildtype phenotype, but upon a subsequent amputation of the regrown head and tail regenerate into a double-headed morphology (unpublished data). When the output weights of the retrained wildtype NN for the posterior head and tail are set to the output weights of retrained double-head NN (Figure 8A), the results are the same as for a double-headed worm, but this is not true for setting output weights of the original parent wildtype NN, which appears to be still wildtype (Figure 8B). For more subtle changes to the retrained wildtype using (Equation 2), the result became closer to the double-headed phenotype in a step-like manner (Figure 9).

(Equation 2)

$$\overrightarrow{w'} = f * d + (1 - f) * \vec{w}$$

where *w* is the output weights for a network, *d* represents the output weights of the double-headed ESN, and *f* is the fraction to add.

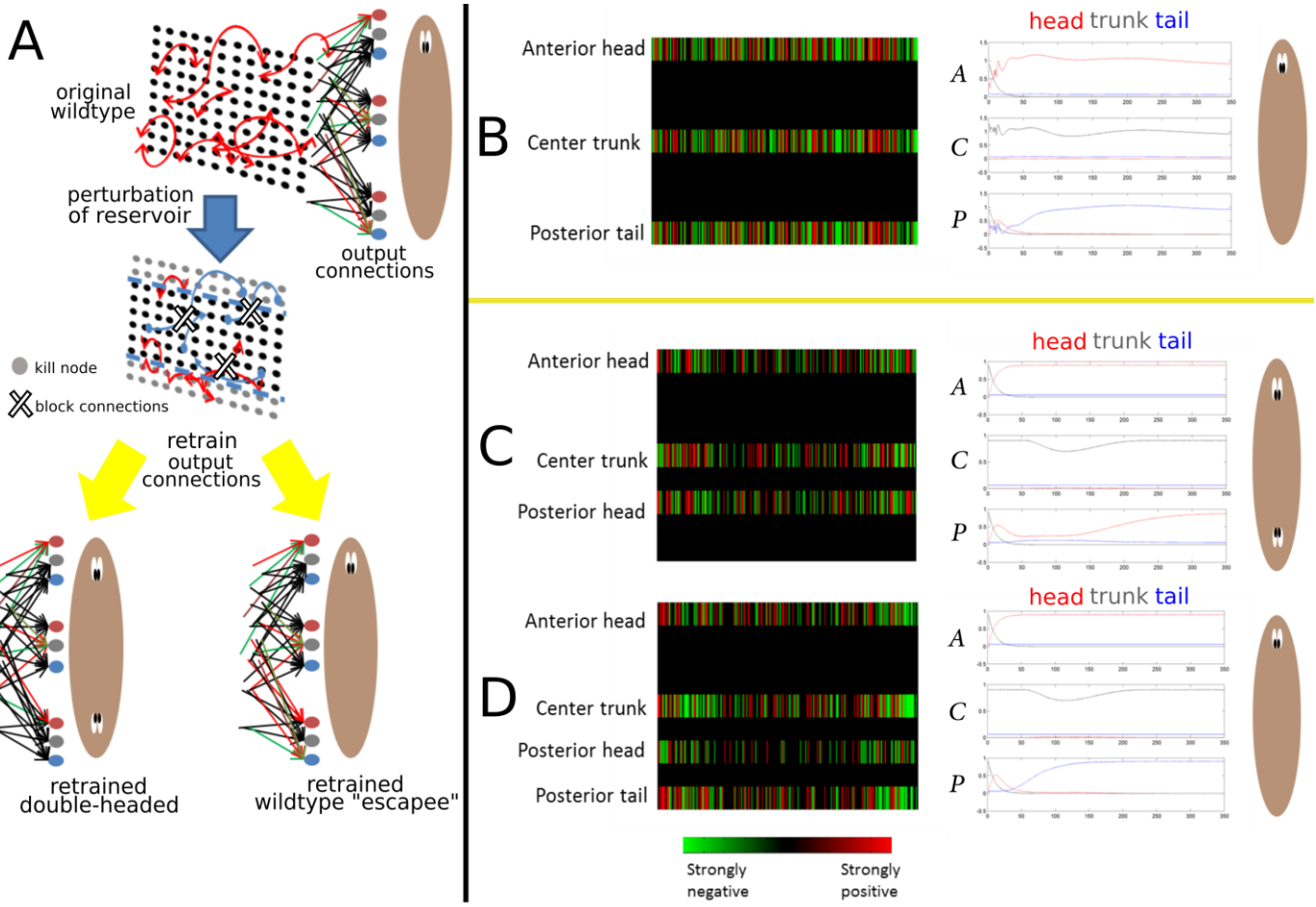

FIGURE 7. Perturbation and retraining of reservoir yields unique detector pattern in between wildtype and double-head. A) We take a network trained on the wildtype input and output pattern with input noise that performs well on the task as expected and perturb the reservoir layer by amputation (blocking of input, internal, and output connections of 40 neurons at the top and 40 at the bottom), and connection blocking of 0.5% of internal connections, and then retrain on the output connections to either double-head positional tissue output or wildtype positional output and examine the newly trained output connections to the reservoir. B) For the original wildtype, the strongest connections are between the reservoir nodes and the anterior head, center trunk, and posterior tail detector outputs as shown by the heat map. The positional tissue output graphs for the 904 time points of simulated wound input show that head tissue has high head in anterior, trunk in center, and tail in exterior. C) The retrained double-head network has strong output for anterior head, center trunk, and anterior tail and for the positional tissue output grows head in anterior and posterior and high trunk

in exterior. We also retrain on D) wildtype which has strong output for anterior head, center trunk, posterior tail and some strong connections in posterior head and has similar positional tissue output as the original wildtype with head anterior, trunk center, and tail posterior.

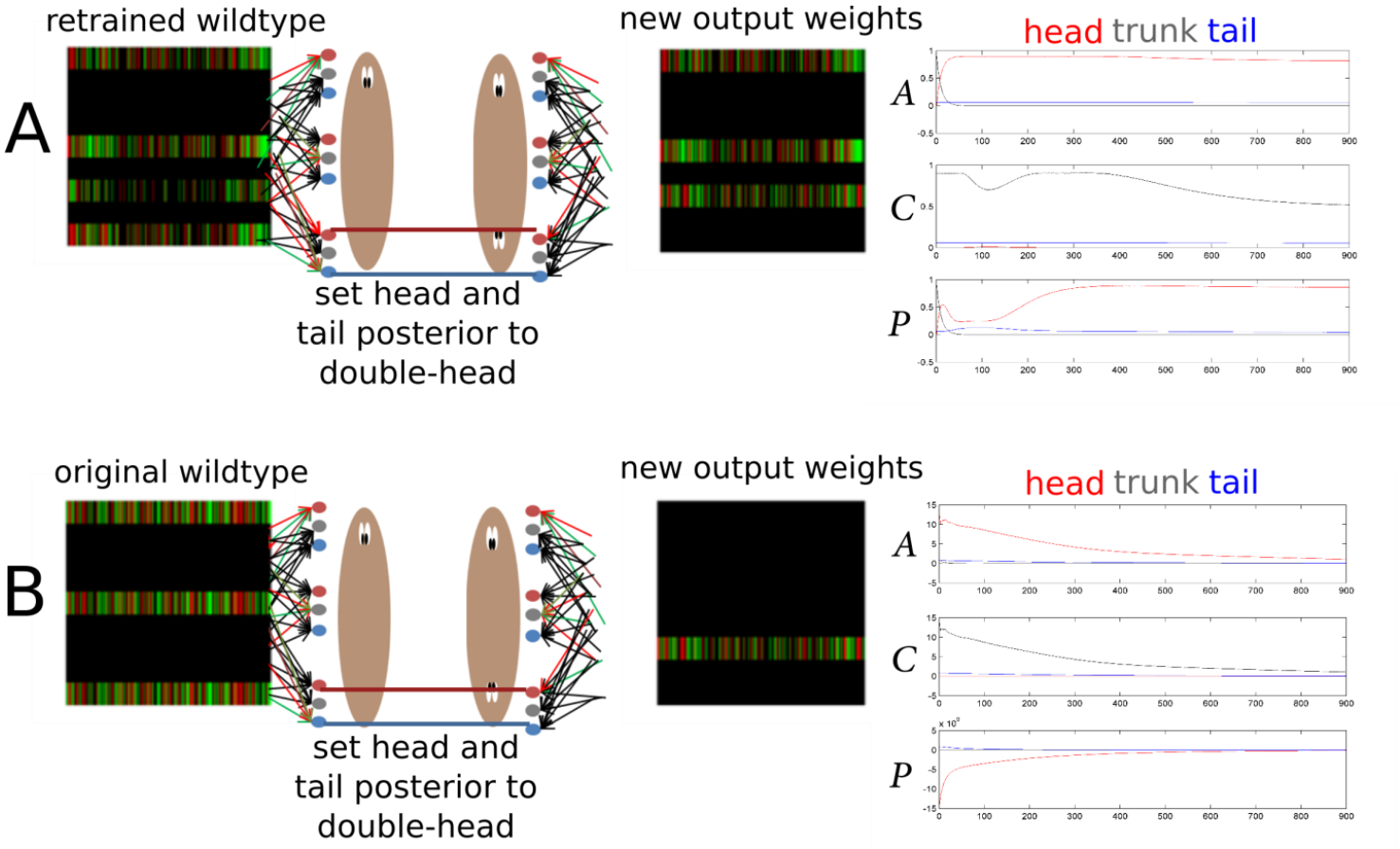

FIGURE 8. Shifting the connections changes output pattern of retrained wildtype to retrained double head. A) For the retrained wildtype, we take set the weights of the posterior head and posterior tail to be the same as the retrained double head, which causes the output weights to look like the double-headed worm, with three bands of strong weights for anterior head, center trunk, and posterior head. Upon the input wound signal generates the output pattern of a double-headed worm on the right, as opposed to the wildtype output. B) We take the original trained network and set the weights of the posterior head and posterior tail to be the same as the retrained double-head network, which causes a novel activity pattern, showing the posterior head to be higher than everything else, which upon the input wound signal generates the output pattern more similar to wildtype than double-headed.

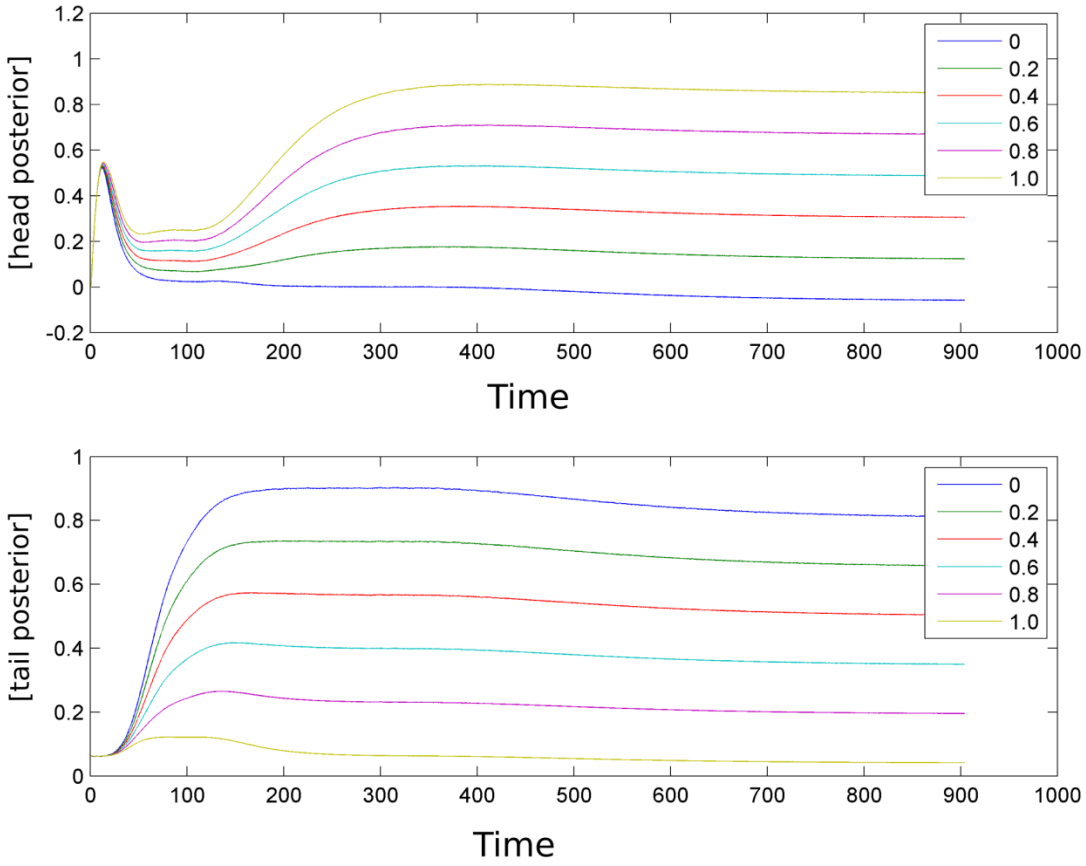

FIGURE 9. Partial changes retrained wildtype posterior head and posterior tail output weights lead to stepwise changes in output. We partially changed the retrained wildtype output weights for the posterior head and posterior tail to be the retrained double-headed output weights, summing the fraction weighted toward retrained double-headed plus the fraction weighted toward retrained wildtype (Equation 2) and measure the output. The posterior head slowly goes from a low concentration at 0 influence of double-headed network, to high concentration for complete (1) double-headed network influence, with the intermediates demonstrating the shift from no head identity to complete head identity. For tail posterior, the relationship is inverse – it begins with a strong growth of concentration of tail in the posterior over time, when the influence of double-headed network is 0 and becomes very low as the network becomes more and more double-headed, until reaches complete (1) double-headed network influence.

5 DISCUSSION

5.1 RESERVOIR MODEL OF REGENERATION

We suggest that reservoir computing is an excellent framework from which to examine the relationship between information processing in a multicellular system relying of bioelectrical communication and regeneration. The lack of formal training of the reservoir allows for the topology to be designed, and the detector readout allows for this to be interpreted for human meaning. The detector could have biological plausibility as well – not as an element of the bioelectrical network, but rather the programmatic readout within cells that leads to directions for regeneration. We formalized the method for analysis of the model and discovered *de novo* phenomena of planaria bioelectrical perturbation, suggesting this model is an important first step in more comprehensive and biologically realistic models of bioelectrical communication in non-neuronal cells for pattern encoding and regenerative remodeling.

5.2 TRAINING WITH NOISY INPUT IMPROVES GENERALIZATION AND ROBUSTNESS OF RESERVOIR

It was discovered that training the network with varied levels of noisy input improved the performance of the network (ability to generate correct output) on novel noisy input (Figure 4) as well as under perturbation (Figure 5). Importantly, we found that it was not just repetition of the input that improved performance, but that to a striking degree, the noisy input was the crucial component in improving the networks' ability to generalize and perform under perturbation. In the brain, stochastic resonance

is a known phenomenon that improves processing, perception, and learning of sensory information [124]. The same principle has been applied to artificial neural networks [125]. Stochastic resonance improves the processing of weak signals. Given the results of our model we suggest that stochastic resonance may also improve the ability of the system to respond to damage, improving the intrinsic plasticity of circuitry systems, as it has been shown that introducing fault into training of ANNs learning classification problems improves their general robustness to other forms of novel faults, the forcing of neuron outputs to zero [126]. This suggests a hypothesis with respect to the mechanism for robustness to perturbation – training with noisy information, which may be absent from non-regenerative systems. It is unclear what mechanism of stochastic might exist as signal inputs *in* vivo to the bioelectric network; this may be instead a property of the internal system or introduced during training and have the same benefits.

5.3 Training with Hebbian rewiring improves Generalization and robustness of reservoir

We found that repeating the input even without noise had a positive effect on the ability of the network to generalize to novel noise (Figure 4B) and be robust to reservoir internal connection blocking (Figure 5B), so to be sure we trained a network with the noisy inputs but without Hebbian rewiring dynamics and found that it did perform worse on generalization to novel noise (Figure 6A) and perturbation (Figure 6B) compared to the network that trained with self-organized rewiring. This is an intuitive result that suggests cells that respond similarly to input are likely to be ‘wired’ together, likely by proximity as well as number and conductivity of electrical synapse connections, and that

this rewiring improves the network's ability to learn complex patterns as well as increase robustness to perturbation.

5.4 PERTURBATION OF RESERVOIR MODEL PREDICTS KNOWN EXPERIMENTAL RESULTS

After our echo state network system was trained to generate positional tissue concentration as output from input of positional wound information, we perturbed the system and retrained on wildtype and double-headed output, to determine if the perturbation would cause any differences in the ability of the system to learn wildtype or double-headed phenotypes. Unexpectedly, the retrained wildtype reservoir output weights were strong for posterior head, suggesting the network had some similar attributes to double-headed trained network (Figure 7). This result is a *de novo* discovery of the planaria "escapee" morphology where seemingly wildtype worms that had been exposed to octanol during their regeneration from trunk fragments will upon subsequent amputation generate double-headed worms. Similarly, changing the bioelectrical connections between the reservoir and posterior head and posterior tail output nodes of the escapee network and the original wildtype network to that of the double-headed network caused the escapee but not the wildtype network to exhibit a double-head output upon wound signal input (Figure 8). This suggests a path from escapee to double-headed worm that is otherwise unexplained from known scientific data.

This theory of the increased strength of head signals in escapee worms could be experimentally tested by better examination of the membrane potential and tissue-specific genetic markers for head and tail for wildtype, escapee, and double-headed

worms to determine if there exist bioelectrical or genetic similarities that suggest the existence of an escapee to double-head regeneration pathway, and to better understand the relationship between *in silico* interpretation and experimental results. Partial changes to the output weights of the nodes by weakly incorporating weights (Figure 9) suggest that the effect of bioelectrical perturbation is cumulative. This evidence suggests that our model of the planaria as a bioelectrical system incorporating wound signal input and positional tissue information output is sufficient to mimic the outcomes of *in vivo* experimentation.

5.5 IMPLICATIONS AND FUTURE DIRECTIONS

Ours is the first model to link neural cognitive models to non-neuronal regenerative systems. The results suggest that an artificial neural network is capable of learning a planaria-like pattern, creating an association between wound input and phenotypic outcomes that also recapitulates *de novo* experimental results that are yet to be understood. We suggest reservoir computing is a particularly good model for bioelectrical communication during regeneration because it separates the system into a memory storage system (reservoir) that contains recurrent connections and a detector program that acts based on reservoir activity. The model and knowledge of planaria may coevolve as more information about the bioelectrical communication becomes available. Future biological experiments that better characterize the bioelectrical properties of planarian somatic cells would significantly advance our model, including:

- Determine gap junctional connectivity for cells in planaria, and whether this connectivity is tissue or positionally specific (i.e. denser gap junctions near

head than tail) and identify maximum and minimum constraints on connectivity, to inform the weights of the echo state network.

- Identify resting potential values across cells at the head, trunk, and tail of wildtype planaria, and identify the constraints (maximum and minimum values) that can be used as the resting potential for the nodes in the echo state network.
- Determine to what extent current bioelectrical perturbation methods like octanol interrupt bioelectrical connectivity. As it stands, our model assumes complete blockage between two cells with estimated rates of perturbation, but it is entirely possible that the blockage between two cells is not complete if only some but not all gap junctions are interrupted.

A first step toward informed values for weights and resting potential of cellular models could be to utilize the conductance models of [127]. As it stands, our reservoir model of regeneration has many potential use cases that have yet to be tested. Some immediate questions include: 1) Do any of the reservoir parameters (spectral radius, reservoir connectivity, see Methods for complete list) have a significant impact on the generalization and robustness of the network, using our assays? 2) What happens to the networks when we perturb reservoirs with small world topology? 3) Can we create a spatial reservoir using a specific mapping of the input nodes to reservoir nodes, such that perturbing areas of the reservoir are akin to perturbing areas of the flatworm? 4) What happens when we adjust the strength of the synapses (weights) between reservoir units? 5) Is there particular set of nodes that are better to remove than others? 6) Can we target perturbation of nodes in the reservoir to get certain behavior? 7) Can

we compare the topology (Hebbian rewiring) of the reservoir for different phenotypic results (no head, no tail, etc)? These experiments on the model will provide testable hypotheses at the bench and provide more data for improving the model.

6 METHODS

6.1 DATA

The data for the recurrent ANN experiments were taken from the time series production of wound, head, trunk, and tail signal across the anterior, center, and posterior regions for the reverse-engineered wild-type planaria gene regulatory network [51]. The double-headed results were taken from the time series for the reverse-engineered wild-type planaria gene regulatory network with octanol treatment. There were 904 time points taken, with 100 variations of the input having random noise from 0.1% to 1% of the standard deviation used for training. For retraining, the networks were trained using the first 350 time points.

6.2 ARTIFICIAL NEURAL NETWORKS

For our recurrent network model of regeneration, we used an echo state network implementation from [67], modified with Hebbian (Oja) dynamics (Equation 1) [128, 129]. For original reservoir experiments, we used a reservoir network with 200 reservoir units, 3 input units and 9 output units, and a feed-forward perceptron as our detector. The internal weights in the reservoir were generated using sparse uniformly distributed random matrix (MATLAB sprand), with a density of 0.05. The input and feedback weights were generated using MATLAB's rand function for uniformly distributed random

numbers, between 1 and -1. The weights of the perceptron detector were trained using the pseudoinverse as was done in [130] (pinv function MATLAB). The learning rate for the Oja rewiring of the reservoir was 0.06, the spectral radius was 0.6 selected by manual trial and suggested in the literature to be optimal under one to maintain the "echo state property" that there is a limit to the time for which an input has an effect on the output [131]. The internal noise level of the reservoir was 1*10^-12, and the feedback scaling was 1*10^-12, both selected by manual testing.

Parameter	Value	Description
Learning rate	0.06	The rate of Oja rewiring of the reservoir.
Spectral radius	0.6	The largest eigenvalue of the internal reservoir matrix, which is important to the "echo state" property of the network. This is modified by the Oja dynamics.
Internal noise level	1*10^-12	The level of uniform noise added when computing the internal states, used for biological reality of imperfect conductance.
Feedback scaling	1*10^-12	Scaling factor to be applied to the output before it is fed into the network.
Reservoir activation function	tanh function	Activation of the firing of the reservoir nodes
Output activation function	identity function	Activation of the firing of the output nodes

6.3 PERTURBATIONS

- Artificial neural network perturbations (removal and connection blocking) were performed according to the methods formalized in [28]. Internal units (neurons) in the echo state networks do not have intrinsic bias values, so modifications were made only to the weights rather than the weights and biases.

  (Equation 3)

  Remove – set the input and output weights and the bias of a specific neuron to 0

  Block connections – set the connection between neuron a and neuron b to be 0 (5), this can be done between both the input to hidden layer connections and the hidden layer to output connections (Fig. 2c).

$$w_{ab} = 0 \qquad \text{(4b)}$$

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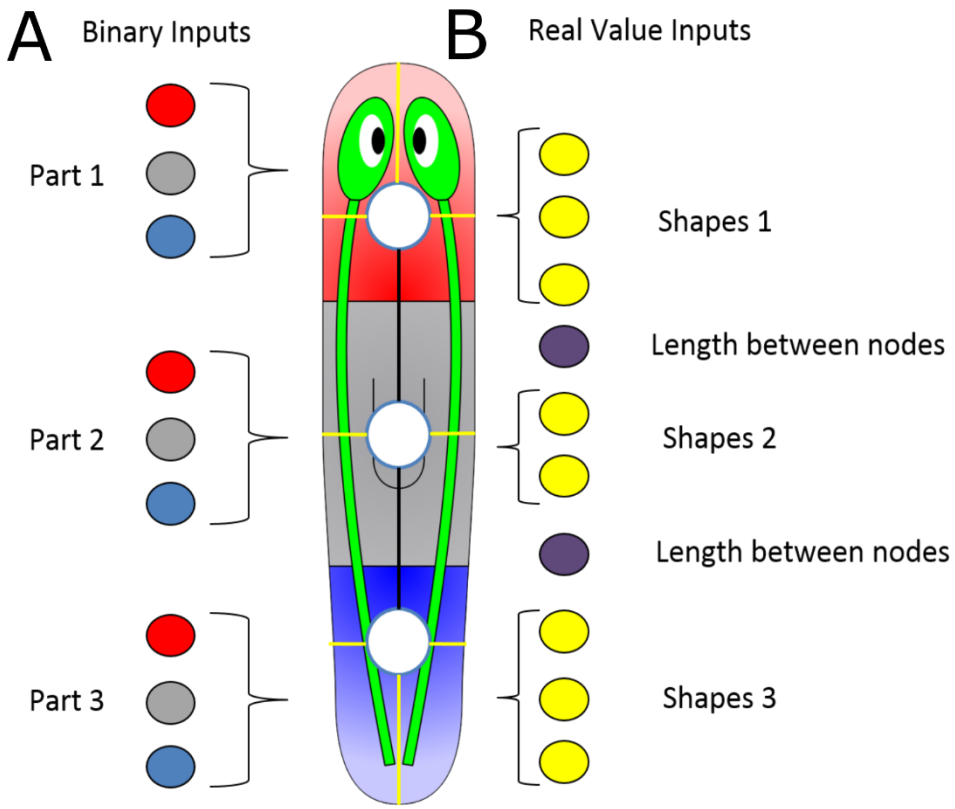
9 APPENDIX I: FEED-FORWARD NETWORKS AS A PLANARIAN REGENERATION MODELS

9.1 FEED-FORWARD NETWORKS LEARN MORPHOLOGICAL/TISSUE INFORMATION FOR CLASSIFICATION AND DETECTION TASKS

We trained a feedforward artificial neural network with 2 hidden layers to learn the task of shape and tissue information to first, using a representation of a flatworm as

input (Figure A1). The data for the feedforward ANN experiments were generated using a simplified version of the graph representation of planaria formalized in Lobo et al [38, 132, 133]. Target morphology was taken to be scalars of the shape and link information (see appendix) with perfect proportions while non-target morphology could be any of problems related to shape, link, or region. For feature detection, the training data were restricted to problems with only one of shape, link, or region such that the network learned to detect a distinct incorrect component. The networks were trained on 400 correct morphology examples, 200 incorrect shapes, 200 incorrect links, and 400 incorrect regions. We trained two different feedforward networks with different tasks for the same input: 1) assign a binary classification to a representation that had all proper tissues and proportions, and one that did not (Figure A2) and 2) recognize the one step that would bring the bad representation closer to a good representation (Figure A3). The network and associated functions are from the MATLAB neural network toolbox [134]. The network was trained with resilient backpropagation, a modified backpropagation algorithm that computes error before taking the derivative so as to provide resilience of the network to outlier training points [135], with mean-squared error to compute backpropagation values. This method was shown to be more resilient to perturbation than the Levenberg-Marquardt training algorithm [28], another common backpropagation method [136]. The networks had 2 hidden layers with 5 and 4 units, respectively, which were chosen as the optimal architecture through manual testing. There were 19 input units and 1 output unit for classification of target and non-target morphology (data not shown) and 19 input units and 19 output units for feature detection of non-target morphology. Networks were successfully able to learn the given

training set as well as generalize to other examples. We then looked at the internal firing patterns for different inputs to determine if we could find specific nodes with identifiable functions (Figure A4) but found though the patterns could be somewhat identified, the individual nodes lacked clear functions. Internal representation was determined to be the singular value decomposition of the firing of the internal nodes for different morphological inputs.

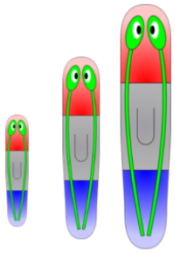


400

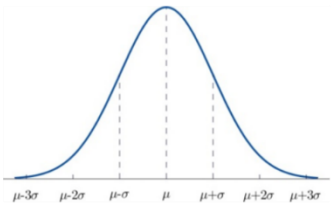
300

400

C Normal worms

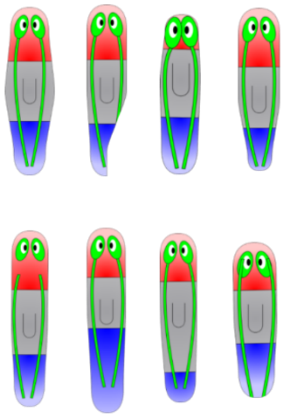


scale=1

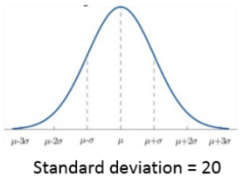


Standard deviation = 0.20

D Proportion problems



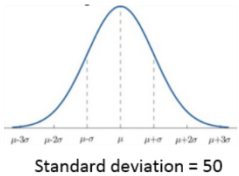
mean=7



Standard deviation = 20

Bad shapes

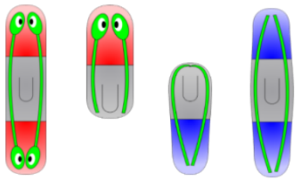
mean=20



Standard deviation = 50

Bad links

E Morphology problems



Pick from:
No head
No trunk
No tail
Double head, trunk, tail
Double head stacked
No tail (stacked trunk)
No trunk (head to tail)
Just head, trunk, tail
200 draw from normal shapes
200 draw from proportion problems

FIGURE A1. Flatworm input representation and training set formation for the binary classification and detection networks. A) Binary inputs represent the presence of head, trunk, and tail information at the anterior, center, and posterior of the work. B) Real value inputs represent shape information and distance between worm sections. Training input is C) 400 examples of normal phenotype worms with different scaling, but same proportions, a standard deviation of 0.2 and mean of 1 D) 300 examples of proportion problems with for the shapes a mean of 70 and deviation of 20 and for links a mean of 20 and deviation of 50, and E) 400 positional tissue problems with positional tissue drawn from specific morphological set.

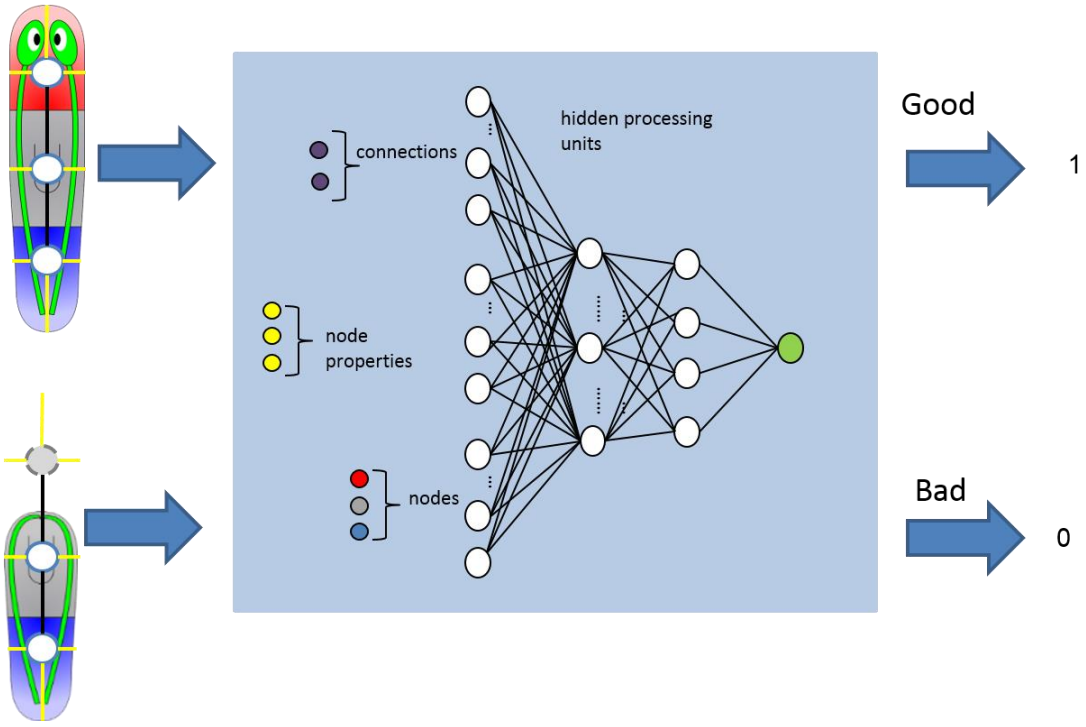


FIGURE A2. Binary classification of planarian input morphology artificial neural network schematic. The input is the binary positional tissue and real-valued shape information, that with a good worm phenotype outputs a binary “1” to signal it as a correct worm and with a bad worm phenotype missing a head outputs a binary “0” to signal it as an incorrect worm. This framework could be seen as “regeneration done” and “regeneration not done” as complement to the problem in regeneration of knowing when anatomical re-patterning is complete.

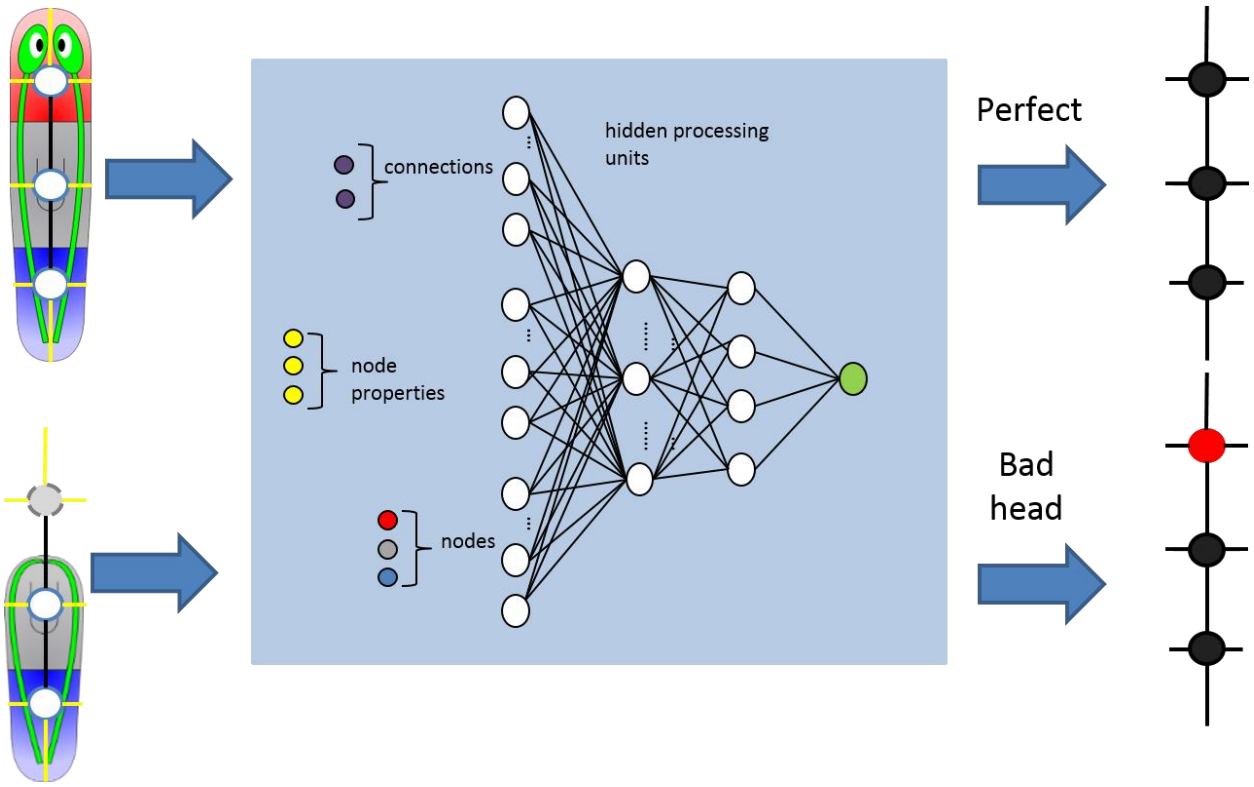


FIGURE A3. Detection of incorrect components of planarian input morphology artificial neural network schematic. The input is the binary positional tissue and real-valued shape information, the same as in Figure S2, but now the network has to detect the area (same as the input) that is incorrect. The network was trained on inputs that were one step away from complete. This network makes suggestions of anatomical edit operations to bring the input closer to a complete worm phenotype.

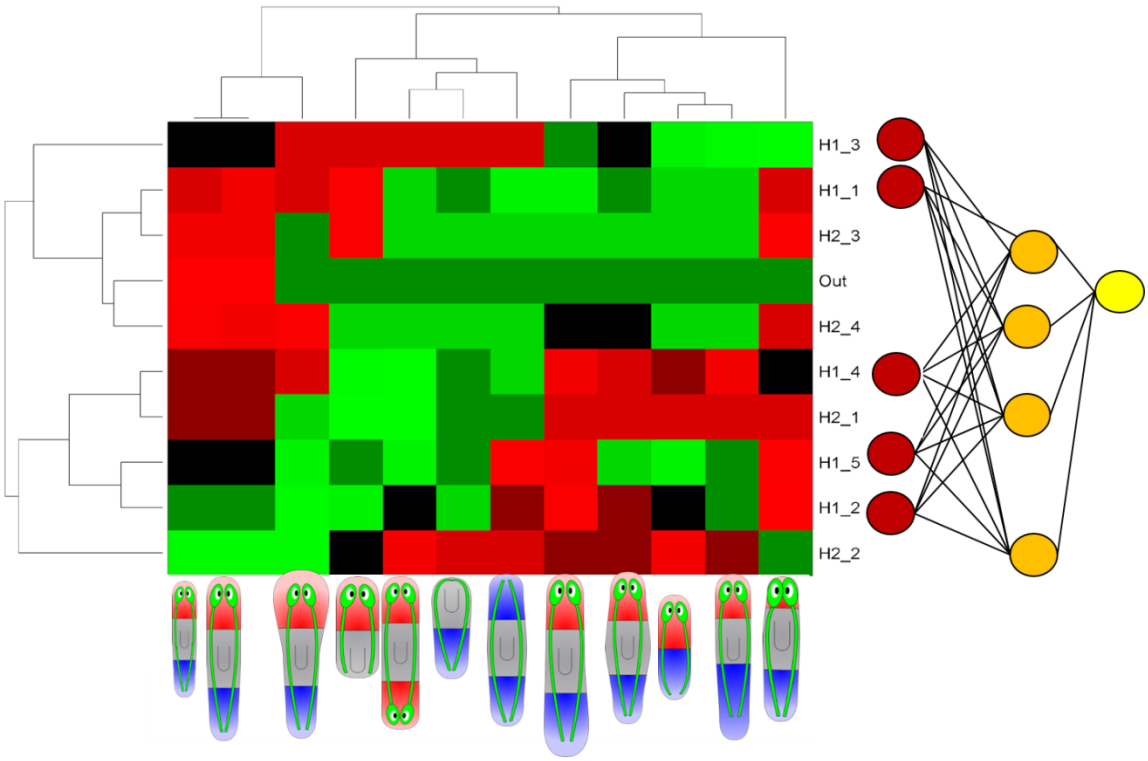


FIGURE A4. Firing output of classification ANN as heat map for different morphological inputs. We examined the firing of neural input (equivalent to function magnetic resonance imaging) for different worm phenotype inputs for the classification network (Figure S2) and found that neuronal function could not be predicted from firing pattern alone, though it was clear that some patterns emerged like for wildtype (two leftmost) phenotype, and some higher level organization can be seen by the clustering of neuron firing pattern using Pearson correlation to two function groups. Neurons H2_2 and H2_4 have almost opposite firing patterns, suggesting functional redundancy exits.

9.2 DIFFERENT NETWORKS LEARN DIFFERENT INTERNAL REPRESENTATIONS

We examined the internal representations for both the binary classification and recognition task networks by using singular value decomposition on the firing patterns for the different morphological inputs. Upon finding multiple distinct internal representations (Figure A5), we decided to test the number of distinct internal

representations. Using k-medoids clustering on the singular value decomposition matrix for the firing patterns of the N different morphologies, we found that there were 2 different clusters, but they were note well separated with an area of high overlap (Figure S6). There was not a significant difference between the accuracy of the 2 clusters and the overlap area.

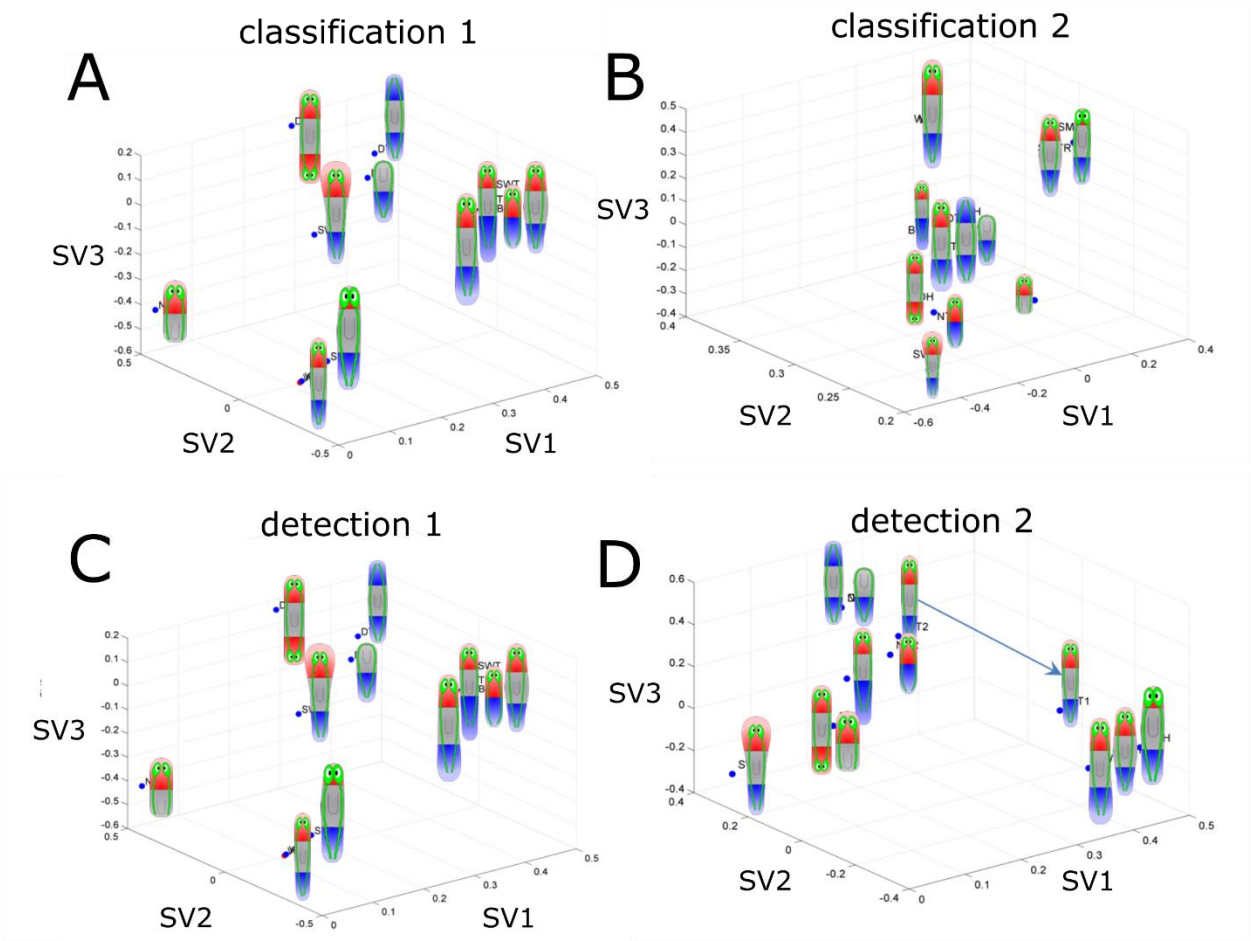


FIGURE A5. Both binary classification and detection networks had very different internal representations for different initial conditions. For two different classification networks A and B trained on the same input data, differing in initial random weights for network components, we have very different internal representations, as

defined as the three highest components in a singular value decomposition of the firing patterns for different inputs (Figure 4). The same is true for detection in C, D though even more striking is the separation of the two wildtype phenotypes, shown by the arrow connecting them, across the internal representation.

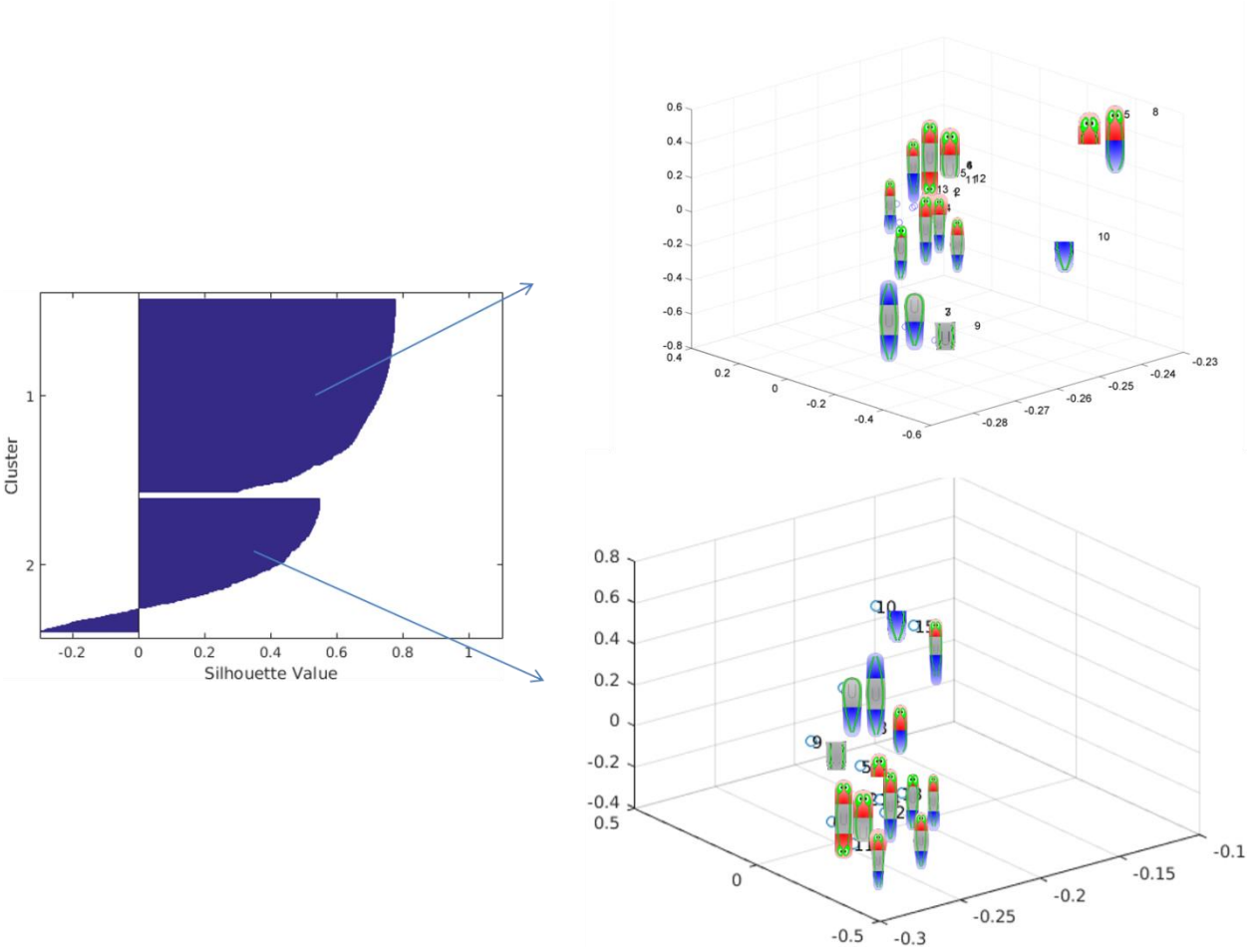


FIGURE A6. Networks are poorly clustered on internal representations. We clustered the internal representation of 100 artificial neural networks using k-medoids with Euclidean distance between the firing patterns of the different worm phenotypes embedded into a 3-dimensional space by SVD. We adjusted the embedding to be closest to another network to account for the possibility of directional and shifting in space. The best clustering was with 2 clusters, but from the silhouette plot (left) it is clear that the second cluster is not distinct (negative silhouette value implies cluster overlap), and the representative networks are unclearly partitioned.

10 APPENDIX II: ADDITIONAL EXPERIMENTS TO RESERVOIR MODEL

We tested the network on several experiments not found in the literature to generate novel hypotheses about the impact of gap junction conductance on regenerative patterning. We found that increasing the conductance of gap junctions by multiplying the connection weights by a constant factor of 1.5 caused the network to have behavior that while in an undefined range of values, had at the end of 900 time points, high anterior head concentration, high center trunk concentration, and high posterior tail concentration (Figure A7). Prior to the network reaching these steady states, the concentration reached sub-threshold values at the 100 time point (Figure A7), which could be hypothesized as a lethal phenotype. Gap junctions have been modified to increase coupled in the heart to treat arrhythmias using the pharmacological agent, antiarrhythmic peptide 10.

We also tested the effect of decreasing conductance of gap junctions by dividing the weights between the reservoir units by a constant factor of 1.5, causing the network to have behavior that ended in the concentration of head, trunk, and tail, at the anterior, center, and posterior all to be 0 (Figure A8). This phenotype has occurred in experiments on regenerating trunk fragments of *S. mediterranea* due to RNAi of *egfr-3* [137], and separately in an experiment with double RNAi of *smg-1* and mTOR complex-1 proteins *tor* or *raptor* as well as RNAi mTOR complex-1 alone [138] , suggesting it is a potential outcome regeneration attempts. Epidermal growth factor receptor kinase [139] activity is known to be required for gap junction closure in mouse oocytes, which implies there could be some relationship between RNAi of *egfr-3* and gap junction communication. The mTOR pathway is also implicated as the effect of a mTOR

inhibitor, rapamycin, results in decreased expression of astrocytic connexin protein Cx43, and furthermore a decrease in gap junction coupling between astrocytes [140].

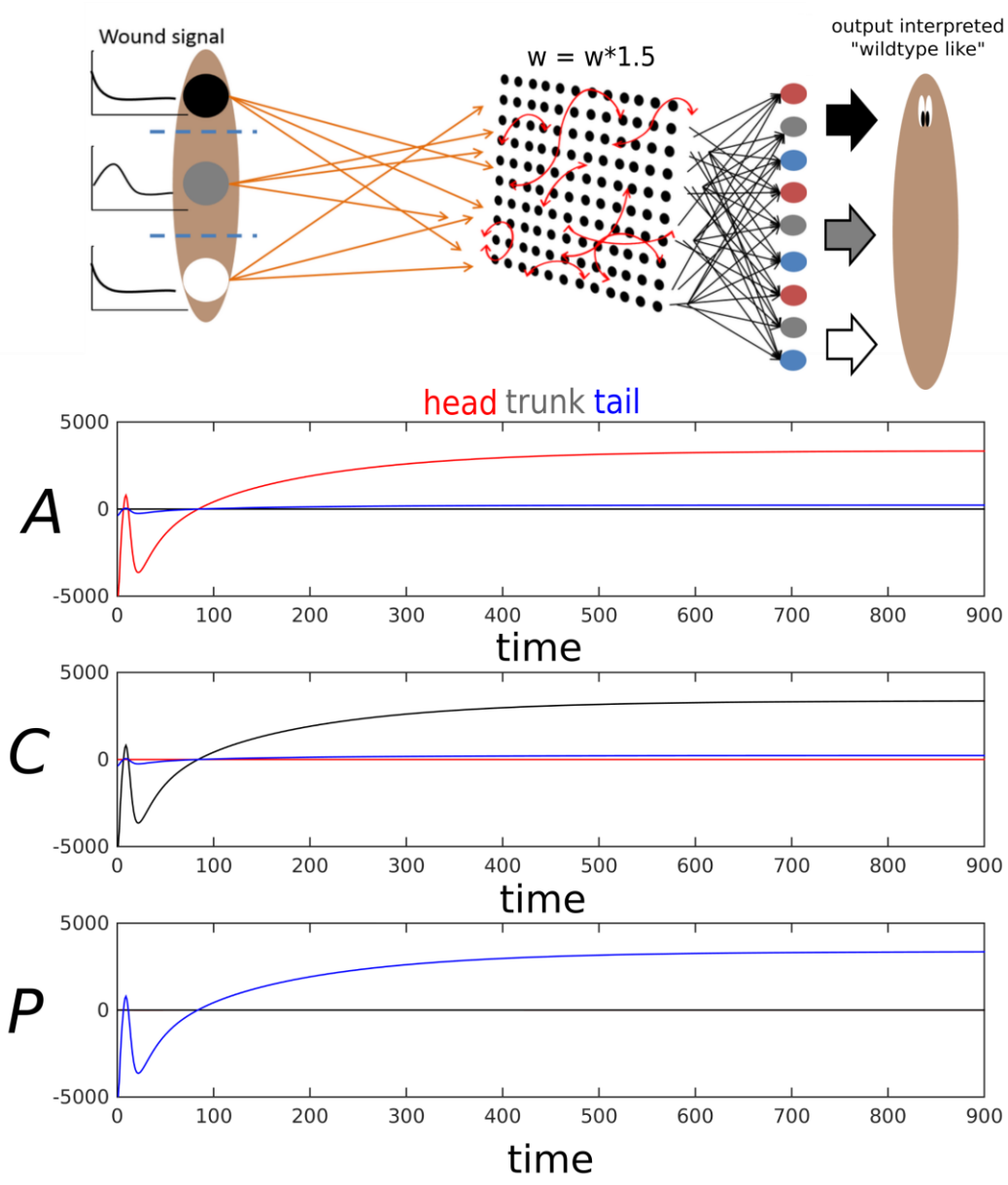


FIGURE A7. Strengthening of all internal synapses predicts phenotypically "wild type" planaria. We strengthened all internal connections (synapses) in the reservoir by half, which would be equivalent to increasing the coupling of all gap junctions. Upon the head-tail regeneration input, this results in a phenotype that has high concentrations of head, trunk, and tail but in the correct spatial pattern.

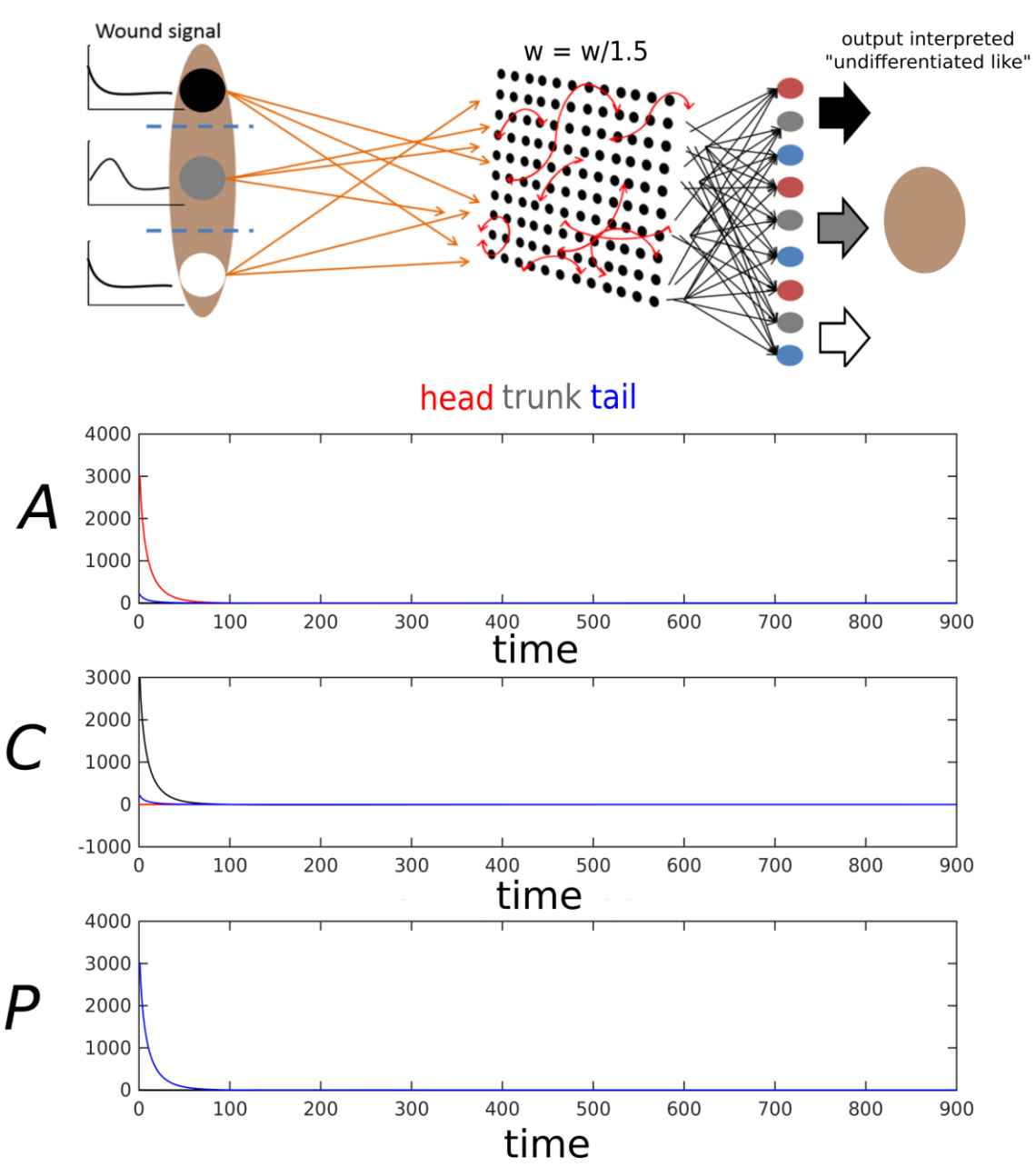


FIGURE A8. Weakening of all internal synapses predicts phenotypically "undifferentiated" planaria. We weakened all internal connections (synapses) in the reservoir by half, which would be equivalent to decreasing the coupling of all gap junction. Upon the head-tail regeneration input, this results in a phenotype that has close to 0 concentrations of head, trunk, and tail suggesting it reverts to all undifferentiated cell type.