

Biological and Simulated Neuronal Networks Show Similar Competence on a Visual Tracking Task

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Abstract—Biological neuronal networks can be embodied in closed-loop robotic systems, with electromechanical hardware providing the neurons with the ability to interact with a real environment. Due to the difficulties of maintaining biological networks, it is useful to have a simulation environment in which pilot experiments can be run and new software can be tested. A simulator for cultured mouse neurons is described, and used to simulate neurons in a closed-loop robotic system. The results are compared to results from a similar experiment using biological neurons.

I. INTRODUCTION

Cultured neurons allow researchers to perform experiments that operate directly on the neurons, without the complications that may be caused by the interacting systems of a living organism. However, cultured neurons are both labor intensive to grow and prone to infection [1], [2]. In order to perform some basic experiments without using biological networks, a simulator was developed to mimic the process of creating biological neuronal networks [3]. A software infrastructure for using these simulated networks interchangeably with biological networks was also developed [3]. The software includes a control program that allows the networks to control a robot arm and receive input from sensors on the arm.

By comparing the behavior of the biological networks and artificial networks in the same experimental configurations, the models used in the simulation can be validated. This paper describes an experiment to test the effect of stimulation on divided networks embodied in a closed-loop robotic system. The system is intended to use a video signal to track a target. Simulated and biological networks display similar distribution of ability to perform the task. Biological networks in control of the arm often display a directional bias in the motion of the arm under their control. It was theorized that distribution of neurons over the sensed area were responsible for this bias. Simulated networks which were created to test whether uneven growth of the culture could contribute to the observed biases in the motion of the robot.

II. CULTURE GROWTH IN VITRO

For the purposes of this work, the neurons under discussion are a network of dissociated mouse neurons grown in a dish called a Multi-Electrode Array (MEA). The MEA consists of a glass plate with an array of electrodes, as shown in Figure 1. Each electrode has a conductive trace that leads to a pad on the edge of the dish, which in turn connects to a very sensitive amplifier. When a neuron sends a signal, its electrical

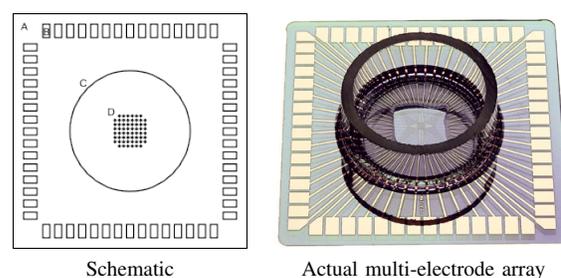


Fig. 1. A. The glass base plate. B. Contacts for connection to amplifier. C. Culture media retaining ring. D. Grid of electrodes to detect neuronal signals. Note that this image is not to scale. The grid of electrodes, in particular, is magnified, as it would not otherwise be visible. The connections between B and D are not shown for clarity.

potential changes, and this change in potential is detected by the amplifier and relayed to the computer. The size of each electrode is close to the size of a single neuron, so neuron firing can be localized to a single neuron or small group of neurons by determining from which pad the signal came.

Fetal mice are used as the cell source because their neurons are still developing and forming connections. The cells are collected and prepared to create a suspension of cells in a media that supports their growth [4]. When the cells are initially added to the culture dish, they are not connected to each other. For most of the first month in culture, the cells build new connections. Starting at around 7 days *in vitro* (DIV) and continuing to around 30 DIV, the connections are not complete, and signaling is dominated by constant, high-amplitude spiking [5].

After the initial period of constant activity, the cells enter a “mature” phase, characterized by sparse bursts of spikes separated by quiet periods. The active bursts may be localized to one region, spread across the network, or propagate from region to region. Cultures used in the control system are in the mature phase of their development.

After 2-3 months of mature activity, the network eventually becomes senescent, and only reacts to stimuli in simple, stereotyped ways [5]. The cells can continue to live for months or even years, assuming that equipment failure or bacterial infection does not kill them [1].

III. SIMULATION OF CULTURE GROWTH

In order to simulate a full MEA, our software models the dispersal of cells over the surface of the MEA, the networking of those cells, and their activity. The first part of the simulation

is deciding the distribution of the cells over an area according to the density of the desired network and the surface area of the MEA plate. The process of determining the cell locations is called “plating.” After the plating simulation has placed the cells, a growth simulation uses the locations of the cells to determine how the individual neurons are connected to form a network. In order to decide which neurons are connected, mathematical models based on the observed networking behavior of real neurons are used. The plating and growth simulation are performed by a body of code called Cultured Neuron Simulator (CNS), which bases its calculations on the same parameters as are available in the plating of biological neurons [3].

The output of the growth simulation is a list of which neurons are connected to each other and what type of neurons they are, excitatory or inhibitory. These are saved as separate files so that the parameters of the resulting network can be modified. For example, specific neurons can be removed to determine their effect on the network, or the network can be divided in half by removing all the connections that cross the center line. Because all of this information is saved in files, it is possible to also keep the original version of a network that has been modified, which is impossible with a biological network.

During execution of the simulation, data is recorded from the neurons located on or near the conductive pads for a specified MEA layout. In order to arrive at a value for the voltage at a specific pad, the voltage at each neuron is reduced according to its distance from the pad, and the sum of the reduced voltage is reported as the voltage at the pad.

IV. ROBOT CONTROL

For the experiments described in this paper, the cultured neurons or the simulated network were placed in control of a robot arm. The arm is a Manus Assistive Robotic Manipulator (ARM), which is a cable-driven 7-DOF arm that was originally designed to be mounted on a wheelchair. To provide input to the neurons from the environment, a video camera was mounted on the end effector of the arm, so that it moves when the arm moves. The activity of the neurons is converted into control signals for the arm by a component of our software infrastructure.

The algorithm used to convert from the voltage recorded at each pad of the MEA to motion commands for the arm is a simplified version of the control scheme from DeMarse *et al.* [6]. DeMarse’s algorithm deals with the spiking activity of the neurons, so it works with both real neurons and any model of neurons that produces physiologically-accurate electrical outputs. Activity in the dish over a short period of time is represented as a 60-item vector, with one activity value for each electrode in the array. The 60-item vector is called the “activation vector”. To calculate the activation vector, the dish is sampled at 1000Hz. For each channel, if a spike is detected on that channel, the activation A at that site is incremented and decayed by:

$$A_n(t_i) = A_n(t_{i-1})e^{-\beta(t_i-t_{i-1})} + 1$$

Activation decays exponentially over time with the decay constant $\beta = 1s^{-1}$. The activations over the sensed area of the MEA are collected in the activation vector V , and normalized to the range 0.0 - 1.0 by applying

$$V_n(t_i) = \tanh(\delta A_n(t_i)) \text{ with } \delta = 0.1.$$

Without normalization, recording sites with very high spike rates can dominate the output, even if the variation of the site in response to stimulation is minimal. The resulting vector of 60 floating-point values is the normalized activation vector for the dish at a specific time, and is updated with every sample from the dish. Every 0.2 seconds the normalized activation vector of the network is compared to a pair of pre-selected activation vectors. The pre-selected vectors are a “right” and “left” vector, with the “left” vector having maximum activation at all pads on the left side of the dish and zero elsewhere, while the “right” vector has maximum activation at all pads on the right side of the dish and zero elsewhere.

The comparison is a simple calculation of Euclidean distance between the left and right vectors and the current activity vector of the network. If the distance from the current activation vector to the left vector is less than the distance to the right vector, the arm will be commanded to move left. Similarly, if the current vector is closer to the right than the left vector, the arm will be commanded to move right. In either case, the difference between the distances must be large enough to overcome a dead band, or the arm is not instructed to move at all. Because it uses two constant vectors for comparison, this system only permits motion left or right, along a single axis. By expanding the selection of vectors used for the comparison, additional degrees of freedom could be controlled.

The images from the video camera mounted on the arm are converted into stimulation signals for the dish. The image is sliced into five strips vertically, and sum of the number of red pixels in the leftmost three and rightmost three strips is calculated. The overlap of one strip in the middle of the image assists with creating a dead band where the system will not move while the target is centered. Overlapping sensing fields have been documented to occur in biological systems, such as the field detection sense of weakly electric fish [7]. If the left side of the image has more pixels than the right side, and the difference is over a fixed threshold, a stimulation signal is sent to the left side of the dish, and vice versa. If the difference between the sides is not large enough, no stimulation is sent. The stimulation is a one-second recording of spiking activity from a biological network [8]. This recording is played back into the network using the analog outputs of the DAQ card in the computer that records the neurons.

V. METHODS

A. Tracking in Biological Cultures

Eighteen biological networks were prepared. The activity of the mature networks is uneven, with some recording sites being more active than others. Different factors could contribute to unbalanced activity in a network. The simplest is that the neurons could have attached to the plate in an uneven pattern,

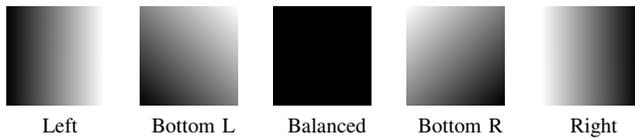


Fig. 2. Images used to control the distribution of cells over the plate, converted to grayscale for print reproduction.

with some areas having no neurons and others having many neurons. It is expected of a biological network that the neurons will not be perfectly evenly distributed over the bottom of the culture dish. If there were more neurons on e.g. the left side of the dish, the left side would be expected to be more active.

To assess whether the networks were balanced or unbalanced, the networks were recorded using Raptor, a software tool developed by the UML Center for Cellular Neurobiology and Neurodegeneration. Raptor generates an 8 x 8 grid of graphs of the voltage at each recording site over the time that the recording was made. These graphs were examined to determine if the voltages at each recording site indicated activity at that site, and if the number of active sites were well-balanced between the left and right sides of the dish. Cultures that were selected based on their balanced activity were mechanically divided and used in the control system.

Each network was placed in control of the arm without stimulation, and then with stimulation. The source of visual input to the system was a tracking target on a pole which was moved manually to attempt to “lead” the arm. The target would be placed as close to the center of the arm’s visual field as possible, and then moved to one side or the other. If the arm moved in a way that would bring the tracking target back into the center of the visual field, the arm was said to track the target. If the arm did not move or moved in a way that would move the tracking target away from the center of the visual field, the arm was said to not track the target.

B. Tracking in Simulated Cultures

In order to determine if the observed bias in the motion of the arm was actually caused by differing levels of neuronal density, a set of simulated networks was created with controlled degrees of unbalance. The simulator accepts as input an image and uses the red channel of that image to set the cell adhesion probability for each possible cell location in the simulated dish. In order to create unbalanced networks, images with a gradient from left to right or right to left were used. To create slightly less unbalanced networks, the gradient was extended from the lower left or right corner of the dish, to the opposite upper corner. This gradient results in lower cell adhesion probabilities in the upper left or right quadrant of each dish, but the “tilt” of the gradient makes the difference between the two sides less severe. The balanced networks were created by using a fully saturated red image.

For each of these five conditions (left, right, lower left, lower right, and balanced), five simulated networks were generated. Each simulated network was divided by removing any connections that went from the left to the right side of

the dish, and vice versa, to mimic the mechanical separation of the biological networks used to control the robot [9].

As with the biological networks, the simulated networks have two stimulation inputs. Detection of the target on the right side of the image triggers stimulation of the right side of the network (and vice versa), which should cause the arm to move towards the stimulated side, bringing the target towards the center of the image. As a result, the system is expected to track the target.

Each of the simulated networks was used to control the robot arm in a simulated world. Instead of the usual camera and arm, a software module accepts messages meant for the arm and uses them to predict the motion of the tracking target that would be detected by the camera, if the arm had actually moved. The software then generates an image to reflect the new position of the target. The simulated view of the world is treated by the other parts of the software as if it was a normal camera image from the video camera. The motion commands to the arm, signals from the simulated dish, and images of the simulated world are all recorded for later analysis.

In order to determine the degree of influence of stimulation on the simulated networks, as well as to determine if the spontaneous behavior of the system as a whole is influenced by asymmetry in the plating of the networks, the simulated networks were run for ten minutes each, both with and without stimulation. If stimulation has a significant influence on the behavior of the network, and so on the actions of the system as a whole, the motions produced while the network is being stimulated will be different from the unstimulated motions of the arm. If stimulation does not produce sufficient change in the behavior of the network, the motion of the arm when stimulus is present and when it is absent will be very similar or the same.

If plating asymmetry produces networks with an uneven balance of activity, then the motion of the arm under the control of simulated networks with more neurons on the left will be towards the left, and towards the right for networks with more neurons on the right. In other words, the overall motion of the arm will be predictive of the imbalance of the neuron plating density. If plating asymmetry does not influence the balance of activity in the networks, then the unbalanced networks and balanced networks will have similar activity.

VI. RESULTS

A. Biological Cultures

Of the networks examined, all of the networks had sufficient activity to cause the arm to move. Four out of 18 networks tracked the target. Out of 57 trials, 5 trials resulted in tracking.

In the biological networks, unstimulated motion of the arm under the control of the network predicts its motion under stimulation, as shown in Table I. If the unstimulated motion of the network displays a strong preference for one direction of motion, then it is likely that the network will display the same bias when it is stimulated. If the network only has a weak bias towards one direction, shown by mostly moving in one direction, but sometimes moving the other direction as

Culture ID	Stimulated	Unstimulated
1	Left	Left
3	Right	Left
5	Left	Left
8	Left	Centered
10	Tracked	Centered
11	Left	Left
13	X	X
14	Right	Centered
15	Centered	Centered
16	Right	Right
17	Right	Right
18	Tracked	Left
19	X	Centered
20	Right	Right
22	Left	Left
23	Left	Left
25	Left	Left
33	Tracked	Left

TABLE I

MOTION OF BIOLOGICAL NETWORKS UNDER STIMULATION AND UNSTIMULATED. X INDICATES AN ERROR THAT RESULTED IN NO MOTION BEING RECORDED. BECAUSE THE UNDERLYING BALANCE OF THE BIOLOGICAL NETWORKS IS NOT KNOWN, IT IS NOT LISTED AS IN TABLE II.

well, then it may move in the other direction or track when stimulated. Biological networks which did not track appeared to have a stronger bias, as expressed by the direction of travel of the robot arm.

Of the networks that did not exhibit tracking behavior, 13 out of 37 runs with stimulation (35.1%) did appear to have some response to the stimulation. Cultures with a strong bias towards one side (as defined above) would be delayed from exhibiting that bias. The motion of the arm would not overcome the existing bias, but it would “hesitate” or make a move opposing the bias if properly stimulated. Another 56.8% of the networks that failed to track moved in a direction that was not immediately attributable to the location of the stimulus, either opposing the stimulus or not moving.

Based on the variety of responses to stimulation, it appears that networks as prepared for this experiment have a gradient of ability. At one end are the few networks that track well, then a larger set of networks that half-track and half-obey their internal bias, and then a larger set of networks with internal biases sufficient to overwhelm the influence of the stimulus.

This spread of relative abilities of the networks to track the target may indicate that some networks are more responsive to stimulation than others. Varying sensitivity to stimulation could be caused by sparsity of sampling points, as the stimulation is only delivered in one location on each side of the dish. If that location is sparsely populated with neurons or sparsely connected, it may not contribute much to spreading the stimulation to the rest of the dish.

B. Simulated Cultures

As with the biological networks, graphs similar to those produced by Raptor were prepared showing the activity of the networks over a 30 second sampling period. Users experienced in the use of Raptor were asked to sort the networks into

Culture ID	Stimulated	Unstimulated	Actual
18:13:45	Left	Left	Bottom Left
18:31:4	Right	Right	Bottom Left
18:45:14	Centered	Left	Bottom Left
19:0:47	Left	Left	Bottom Left
19:17:12	Left	Left	Bottom Left
19:35:0	Centered	Right	Bottom Right
19:49:41	Left	Left	Bottom Right
20:5:22	Right	Right	Bottom Right
20:21:20	Right	Right	Bottom Right
20:37:11	Centered	Left	Bottom Right
20:54:50	Centered	Centered	Left
21:10:25	Centered	Centered	Left
21:27:27	Left	Left	Left
21:42:15	Right	Right	Left
21:59:42	Right	Right	Left
22:14:27	Centered	Right	Right
22:32:30	Right	Right	Right
22:49:34	Right	Right	Right
23:23:48	Centered	Left	Right
23:6:13	Right	Right	Right
23:38:58	Right	Centered	Balanced
23:57:1	Centered	Right	Balanced
0:18:45	Centered	Right	Balanced
0:24:40	Right	Right	Balanced
0:42:40	Centered	Right	Balanced

TABLE II

FOR THE SIMULATED NETWORKS, THE MOTION LISTED IN THE STIMULATED AND UNSTIMULATED CONDITIONS DESCRIBES THE BEHAVIOR OF THE NETWORK WHEN IT WAS CONNECTED TO THE ARM WITH AND WITHOUT FEEDBACK FROM THE CAMERA. THE ACTUAL DISTRIBUTION OF THE NETWORK IS LISTED IN THE LAST COLUMN.

left, right, and balanced groups. Out of the 25 networks, 20 are either left or right biased, with 5 each of left, right, lower left, and lower right distributions. The remaining 5 are balanced. The experienced users were able to correctly classify 16 out of 25 (64%) of the networks as left or right biased. The severely unbalanced networks were easier to classify, with 9 out of 10 (90%) identified correctly. Balanced and slightly unbalanced networks were easily confused. Out of the 10 slightly unbalanced networks, 5 (50%) were identified correctly, and the other 5 were misidentified as balanced networks. This indicates that the simulated networks did have the desired balance or lack of balance, as their activity as classified by experienced users matched the expected activity based on the input images.

The unstimulated motion of the network does usually match the direction that the network will move the arm when stimulated, but does not appear to accurately predict the imbalance imposed on the network during its development. In 16 (64%) of the 25 simulated networks, as listed in Table II, the motion of the arm while unstimulated matched the motion of the arm under stimulation. In 13 (52%) of the simulated networks, the motion while unstimulated matched the underlying distribution of the network. The others did not match, and so the unstimulated motion only had approximately a 50% chance of predicting the bias the network was expected to have due to uneven seeding. Similarly, in 12 (48%) of the simulated networks, the motion under stimulation matched the underlying bias of the network. Again, this gives the stimulated

motion only a half-chance of predicting the actual bias.

Without stimulation, eight networks displayed a left bias when unstimulated. Five of them were left or left-bottom unbalanced, so 62.5% of the left-moving networks had a leftward bias in their plating, and only 37.5% had any other bias. If the biases were exhibited randomly, 80% of the networks that moved left would be expected to be in non-left-biased configurations, as 80% of the network configurations are not left-biased. It is more likely that a left-biased network will display a leftward bias in its unstimulated motion.

Fourteen networks moved right in the unstimulated condition. Of those, seven (50%) were right or right-bottom unbalanced, as opposed to the 20% that would be expected if the biases were displayed randomly. It also accounts for 70% of the right and right-bottom biased networks, so again, the unstimulated bias does appear to predict the underlying bias of the network.

Five of the networks that moved right were created as balanced networks. The remaining three networks remained centered, but only one of these networks was a balanced network, the other two were left-biased. Because the sensed area of the MEA is relatively small compared to the total dish area, it is possible that while the overall distribution of the cells in the simulated MEA was as defined by the input image, the actual bias over the sensed area was different, due to stochastic elements of the simulated plating process. This would result in ostensibly “balanced” networks that nonetheless display a slight bias in the absence of any stimulus to counter it.

When stimulation was applied, five networks moved left, of which four (80%) were in one of the left-biased configurations. The remaining network was in the bottom-right configuration, but had displayed a leftward bias when unstimulated as well. It is possible that the configurations that biased the cell distribution towards the left or right bottom corner were close enough to evenly balanced over the sensed area that the intended bias was not strongly expressed.

Of the 10 networks that moved right when stimulated, 5 were in one of the right-biased configurations. Because there are two different left- and right-biased configurations for the networks, but only one balanced configuration, the chance of a randomly selected network being left-biased is 40%, as is the chance of it being right-biased, while the chance of it being balanced is 20%. Getting a 50% match of motion in a particular direction and underlying bias is therefore better than chance.

The 10 networks that tracked under stimulation were in several different configurations. One was bottom-left biased, two were bottom-right biased, two were left biased, two were right biased, and three were balanced. In all but two cases, the networks had exhibited unbalanced behavior while unstimulated, so the tracking was a change in response to the stimulation. This shows that the stimulation both affects the behavior of simulated networks, and can overcome a previously exhibited bias.

As with the biological networks, some simulated networks are more amenable to stimulation than others. Plots of the

motion of the arm after the stimulated and unstimulated runs indicate that some of the networks had a much larger change in behavior than others in response to stimulation. In general, these are the networks that had a preferred direction of motion while unstimulated, but became centered when stimulation was applied. Of the 9 networks that became centered under stimulation, 3 of them were balanced networks, constituting 60% of the balanced networks, with the remaining 6 representing 30% of the unbalanced networks. This indicates that while balanced networks are more likely to track under stimulation, some unbalanced networks may also be able to track a target.

The responsive networks had large differences in their motion between the stimulated and unstimulated conditions. Unresponsive networks, on the other hand, had very small degrees of difference between their actions when stimulated and their actions when unstimulated. Overall, 8 of the 25 networks (32%) changed their direction of movement between the stimulated and unstimulated runs. Of these networks, 3 were balanced, 3 were weakly unbalanced, and 2 were strongly unbalanced. There are only 5 balanced networks, so the balanced networks were more likely to change their direction of movement under stimulation than the weakly or strongly unbalanced networks. A balanced network would be expected to have balanced activity, and so the stimulation would not have to overcome a pre-existing bias in the activity of the network.

VII. COMPARISON

The mechanism for creating unbalanced simulated networks results in a change in the electrical activity such that experienced users could correctly classify 16 out of 25 (64%) of the networks as left or right biased by examining the recorded voltage of the networks over a short period. The voltage records were presented in the same format that is used to determine if the activity of biological networks is balanced. From this, we can conclude that the methods used to create unbalanced simulated networks do result in similar activity similar to that of biological networks.

The pre-existing unbalanced activity of artificial networks does not predict the behavior of the full system when stimulation is absent. Only 25% of the networks displayed the same bias in their motion as was imposed on the distribution of neurons within the network. The effects of a gradient of neuron density over the entire culture area may be overwhelmed by local variation between contact points, because only a small number of the neurons located near the center of the dish are sampled by each contact point. As a result, a network that is overall biased with higher neuron density on the left may actually display more activity on the right side of the sampled area and vice versa.

Stimulation does have an effect on the signaling of both artificial and biological networks, and is reflected in the motion of the arm. Only 32% of the simulated networks changed their direction of movement under stimulation, but this closely matches the 37% of the biological networks that changed their direction of movement under stimulation. From this result, it

appears that the percentage of networks that are amenable to stimulation in this system is roughly the same between biological and simulated networks.

A pre-existing unbalanced activity in unstimulated networks, whether biological or artificial, does predict their behavior under stimulus. For 16 of the 25 simulated networks (65%) the motion of the network under stimulation matched the direction of motion of the network without stimulation. Due to technical difficulties and culture mortality, the data for biological networks in Table I is somewhat more sparse than the data for simulated networks. Of the 16 runs where useful data was recorded, 10 (62%) resulted in the motion of the arm under stimulation matching the motion of the arm without stimulation. The percent rate at which the prediction holds is very similar to that exhibited by the simulated networks, which indicates another point of similarity between the simulation and biological networks.

VIII. DISCUSSION

These points of similarity between the simulated and real neurons did not require exceptional tuning of the simulator to produce. The values used to configure the simulator are set to the same values used to create the biological networks, in terms of cell density, neuron types, etc. These similarity between biological networks and simulated networks in terms of predictability of behavior under stimulation and amenity to stimulation indicate that it is possible to simulate the activity of certain configurations of networks at a phenomenological level. The simulated networks are thus simulations of a broad class or type of biological network. However, a more interesting problem would be developing a simulation of a specific individual network. As previously mentioned, the neuron types and connectivity of each cell in the simulated network are recorded in a file and can be edited. By varying the connections and connection strengths of a simulated network, it could be modified to bring its activity in line with the observed activity of a particular biological network. A number of metrics exist for assessing the similarity of two networks, such as inter-recording-site correlations or Center of Activity Trajectory (CAT) [10]. Culture metrics could be used as a way to assess the results of tuning a simulated network in comparison to a biological network. As a result, machine learning approaches could be used to automate the process of developing a simulation of an individual biological network.

Unfortunately, the simulated networks developed by such an algorithm would not likely be an identical match for the connectivity and neuron placement of the original biological network. If multiple permutations of connections between neurons can give rise to similar signaling patterns, then the simulated network can display similar signaling to the biological network without having the same connectivity. Because the real and simulated networks' connectivities would likely differ, a local perturbation, such as the division of the network used in the experiments described in this paper, would affect the two networks differently.

Even without the ability to simulate specific networks, simulating a class of networks has some promise for accelerating work with biological networks. Because the simulated networks are not susceptible to disease and require little time to prepare, vast numbers of them can be prepared and the data from them can be automatically analyzed. As neuronal network simulators improve, their output can be used to guide the selection of parameters for biological networks into interesting areas of the configuration space. This guiding would save time, money, and effort in the creation of populations of networks for research. In turn, the results from biological networks would be used to inform the development of the simulator.

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