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Neurosimilator for Undergraduate Biophysics and Neurophysiology Courses

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Stringent animal welfare principles are forcing undergraduate instructors to avoid the use of animals. Therefore, many hands-on lab sessions using laboratory animals are progressively replaced by computer simulations. These versatile software simulations permit the observation of the behavior of biological systems under a great variety of experimental conditions. While this versatility is important, computer simulations often work even when a student makes wrong assumptions, a situation that poses its own pedagogical problem. Hands-on learning provides pupils with the opportunity to safely make mistakes and learn organically through trial and error and should therefore still be promoted.

We propose an electronic model of an excitable cell composed of different modules representing different parts of a neuron - dendrites, soma, axon and node of Ranvier. We describe a series of experiments that allow students to better understand differences between passive and active cell responses and differences between myelinated and demyelinated axons. These circuits can also be used to demonstrate temporal and spatial summation of signals coming to the neuron via dendrites, as well as the neuron coding by firing frequency. Finally, they permit experimental determination along with theoretical calculations of important biophysical properties of excitable cells, such as rheobase, chronaxie and space constant.

This open-source model has been successfully integrated into an undergraduate course of the physiology of excitable cells and student feedback assessment reveals that it helped students to understand important notions of the course. Thus, this neuromorphic circuit could be a valuable tool for biophysics and neuroscience courses in other universities.

Key words: rheobase; temporal and spatial summation; refractory period; time constant; space constant; strength-duration relationship; chronaxie; frequency coding; signal propagation velocity; education

Contemporary biomedical research and teaching are both constrained to follow measures to assure the welfare of animals used. Strict application of 3R principles (refinement, reduction and replacement) forces undergraduate teaching in medical universities to abandon the use of vertebrate laboratory animals. Practical courses using living objects are progressively replaced by manipulations with relevant computer models, since they permit observation of biological systems under various experimental conditions. While versatility is important in the teaching environment, computer simulations take over many important parameters and experimental procedures by default. Thus, this experience remains virtual, which rationalizes why many medical training facilities, instead of computer simulations, opt for replicas that resemble real biological objects as closely as possible. Clearly, hands-on learning endows students with the opportunity to safely make mistakes and to learn organically through trial and error with some acceptable limits.

The nervous system encodes information in the form of action potential trains and subthreshold membrane potentials. The information processing units, neurons, have multiple inputs (synaptic knobs on dendrites) and unique output (the axon). Basic properties of neurons include all-or-none output excitation, inhibition, threshold value, refractory time, temporal and spatial summation (Hille, 2001). In the 1950s, Hodgkin and Huxley argued that time courses of electrophysiological and purely electrical phenomena are very similar (Hodgkin and Huxley, 1952). They've also shown that the behavior of excitable cells can be accurately reproduced on the basis of nodal analysis of equivalent electrical circuits of neuronal membrane (Hodgkin and Huxley, 1952). Analog neural engineering gained important insights from this work and many scientists were able to create real-time electronic prototypes of single excitable cells and synapses (van Bergeijk and Harmon, 1960; Malmivuo and Plonsey, 1995; Gerstner and Kistler, 2002; Indiveri et al., 2011). In this article we describe a novel electronic model of an excitable cell. The proposed circuitry represents an original analog approach. It has been specifically elaborated for teaching purposes, in which individual electronic components simulate as close as possible different ionic conductances of a nerve fiber. In comparison to educational analog models that simulate Hodgkin-Huxley model (Koch and Brunner, 1988; Rutherford et al., 2020), it keeps a high level of accuracy and biological plausibility, while being much simpler and compact. Accordingly, it is a real-time model as opposed to microcontroller-based hardware developed for neurophysiology/biophysics teaching (Petto et al., 2017; Burdo, 2018; Baden at al., 2018; Land, 2014; Land, 2016a, 2016b). Rich and tunable neuronal behavior of the model as well as its unprecedented accuracy in reproducing the shape of action potentials (as seen in cortical neurons or in squid giant axon) allowed us to name it "Neurosimilator".

The Neurosimilator is composed of modules representing different parts of a neuron: dendrites, soma, axon and node of Ranvier. Modular construction of
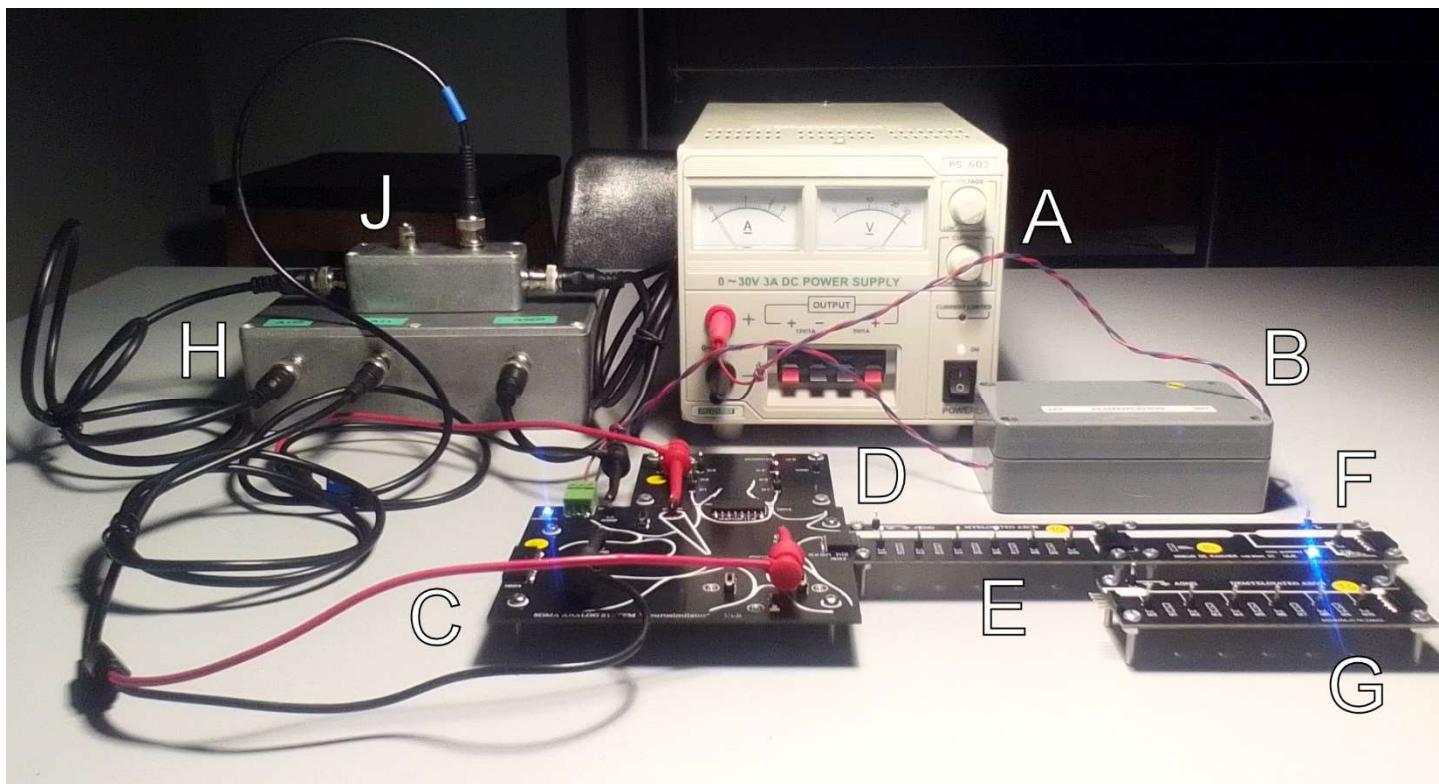
Figure 1. General View of Neurosimilator. *A* – power source, *B* – power adapter, *C* – soma, *D* – dendrites, *E* – myelinated axon, *F* – node of Ranvier, *G* – demyelinated axon, *H* – AD-DA interface, *J* - BNC hub. BNC hub is connected to analog output AO0 and to one of the analog inputs of the interface. Black hooks of coaxial cables are connected to the reference point (ground). Red hook of the cable coming from BNC hub is connected to the stimulation electrode. Red hook of another cable connects soma to the second analog input of the interface.

The presented circuit provides flexibility - distinct circuit parts are functionally separated, so that each module can be modified to change a particular neuronal property, if needed. This is an important advantage in teaching a biophysics/ neurophysiology course. We describe manipulations with the circuit performed for a number of academic years by undergraduate students at the Université libre de Bruxelles. These inquiry-based experiments allow students to better discern differences between passive and active cell response and to figure out major factors responsible for the generation of action potential. These manipulations demonstrate passive behavior of myelinated and demyelinated axons, provide insights into temporal and spatial signal summation in the neuron as well as to neuron coding by firing frequency. They also allow experimental determination of important biophysical properties of excitable cells, including rheobase and chronaxie together with time and space constants. With additional circuit boards that can be connected to soma, different oscillatory patterns can be observed and studied.

MATERIALS AND METHODS
Description of Neurosimilator Modules
The system consists of several subunits, which are assembled as shown in Figure 1 to form a complete model of neuron. These modules are the soma, a dendritic membrane, two types of axons (myelinated and sclerotic, i.e., demyelinated) followed by the node of Ranvier. The electronics are driven by a power supply unit providing positive and negative rails referenced to floating ground generated from a standard 30 V laboratory power supply, which is converted to stabilized 24 Vdc and then split to +12 V and -12 V referenced to the virtual ground. The voltage source is connected to the soma only, from which the power is distributed to each point in the circuit modules via connectors and headers between them. Detailed schematics of the power source and neuron modules can be found in the Supplementary Information files.

Axon
The passive axonal membrane is enacted by RC-units consisting of 200 pF capacitor (C_a) in parallel with 10 MΩ resistor (R_a) in case of a myelinated membrane, or 10 nF capacitor in parallel with 1 MΩ resistor in case of a demyelinated membrane. Individual membrane units are interconnected by longitudinal resistances (R_{axo}=120 kΩ), mimicking resistance of the axoplasm, a column of electrolyte enclosed by the axonal membrane. One axon contains an array of ten such elements in cascade. The axon is connected to the node of Ranvier, whose input is buffered
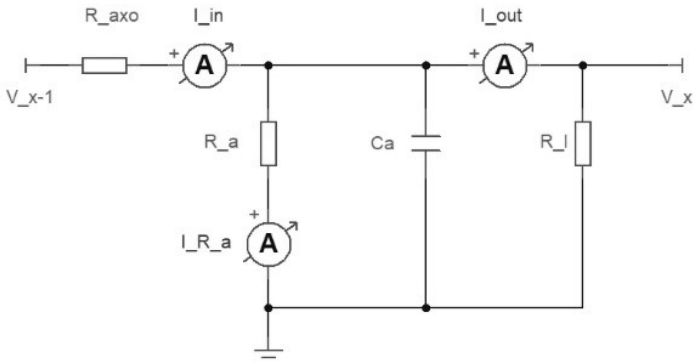
Figure 2. Last Module of an Axon with a Continuity Compensation Using a Load Resistance. Amperemeters are introduced to indicate points of measurement of corresponding current.

to ensure that the node does not electrically charge the axon, i.e., the signal is propagated only in one direction. It should be noted that the presence of high input impedance voltage follower amplifier between the axon and node of Ranvier breaks circuit continuity, so that no axoplasmic current (I_{out}) flows out of the last axon unit. This creates deviation from the theoretical cable properties of the axon. It is possible to avoid this problem using different dimensioning of the modules, however in order to solve it for the dimensioning above we introduced a load resistance (R_l) at the end of axon as shown in the Figure 2.

To calculate the value of this resistance we employed the reasoning as follows. We know that voltage at the module *x* (V_x) of a discrete axonal circuit is calculated as:

$$V_x = V_{x-1} \cdot e^{-\frac{1}{\lambda}} \qquad \text{eq.1}$$

where $\lambda = \sqrt{R_a/R_{axo}}$. The Ohm's law suggests that current entering the module x is:

$$I_{in} = \frac{V_{x-1} - V_x}{R_{axo}} \qquad \text{eq. 2}$$

while the leak current flowing out to the ground through the membrane at the module *x* is:

$$I_{R_a} = \frac{V_x}{R_a} \qquad \text{eq. 3}$$

since $V_{ground}=0$ V. Finally:

$$I_{out} = \frac{V_x}{R_l} \qquad \text{eq. 4}$$

because non-inverting input of the voltage follower amplifier is at zero Volts. Kirchhoff's current law indicates that in a stationary state (when capacitor C_m is fully charged) or in a pseudo-stationary state (when capacitor is not charging momentarily, such as at the peak of action potential):

$$I_{out} = I_{in} - I_{R_a} \qquad \text{eq.5}$$

Solving equations to find R_l gives:

$$R_l = \frac{R_a \cdot R_{axo}}{R_a \left(e^{\sqrt{R_{axo}/R_a}} - 1\right) - R_{axo}} \qquad \text{eq.6}$$

In case of myelinated membrane, this load resistance amounts to R_l=1.16 MΩ. In case of demyelinated axon such resistance (amounting to R_l =434 kΩ) is not necessary because the capacitances of 10 nF provide the required impedance to the ground for loading at the frequency of observed action potentials (i.e., RC filtering), which compensates accurately for the deviation above. In the model circuit, two compensation approaches are possible: either by fixing R_l in parallel to R_m of the last axon RC unit or by replacing R_m of this last unit by an equivalent resistance of R_m and R_l in parallel (R_{eq}=1.05 MΩ). A continuity test on mounted PCB gives the following equivalent resistance and capacitance values of axons, when they are measured using multimeter between the output of the last axon module and the ground: for myelinated axon: ≈645 kΩ and ≈400 pF; for demyelinated axon: ≈292 kΩ and ≈8 nF. It should be noted that only passive propagation of the signal with a waveform of action potential can be studied with these axons.

Dendrites
The dendritic membrane serves to deliver synaptic currents to the soma. In general, it has a structure and properties similar to the demyelinated axonal membrane with somewhat smaller space constant because of the smaller radius of dendrites compared to the axon. Nevertheless, since the dendrites are much shorter than the axon, they should deliver significant portion of synaptic currents before they are largely attenuated. Each RC unit in arrays in this case is formed by a 10 nF capacitor in parallel with a 150 kΩ resistor and these units are separated by longitudinal resistance R_{axo} (82 kΩ). One dendrite module contains two separate branches formed by arrays of three RC-units in cascade. The summation of dendritic currents from these branches is realized using two identical resistances connected to a common point at the positive entry of non-inverting amplifier with a gain of 2, which also ensures unidirectional flow of signals - from dendrites to soma only.

The Soma and Node of Ranvier
The proposed electric schematics of the neuron's soma depicts a novel circuitry that has only a slight resemblance to already available topologies. The block diagram and simplified schematics is presented in the Figure 3.

Passive properties of the soma are determined by membrane capacitance (C_m=10 μF) and membrane leak resistance (R_m =1 MΩ). It is evident that in the absence of a power source, the soma behaves as a passive RC filter. Since all types of currents flow in parallel to membrane capacitance C_m, the membrane voltage of the soma follows the charging and discharging of C_m. Voltage-sensitive sodium current with a positive feedback activation is generated using an operational amplifier with a gain of 2 and with a resistance, diode, and capacitance mounted in its positive feedback loop. The diode sets the threshold for the activation that is achieved progressively at approximately 0.7 V.

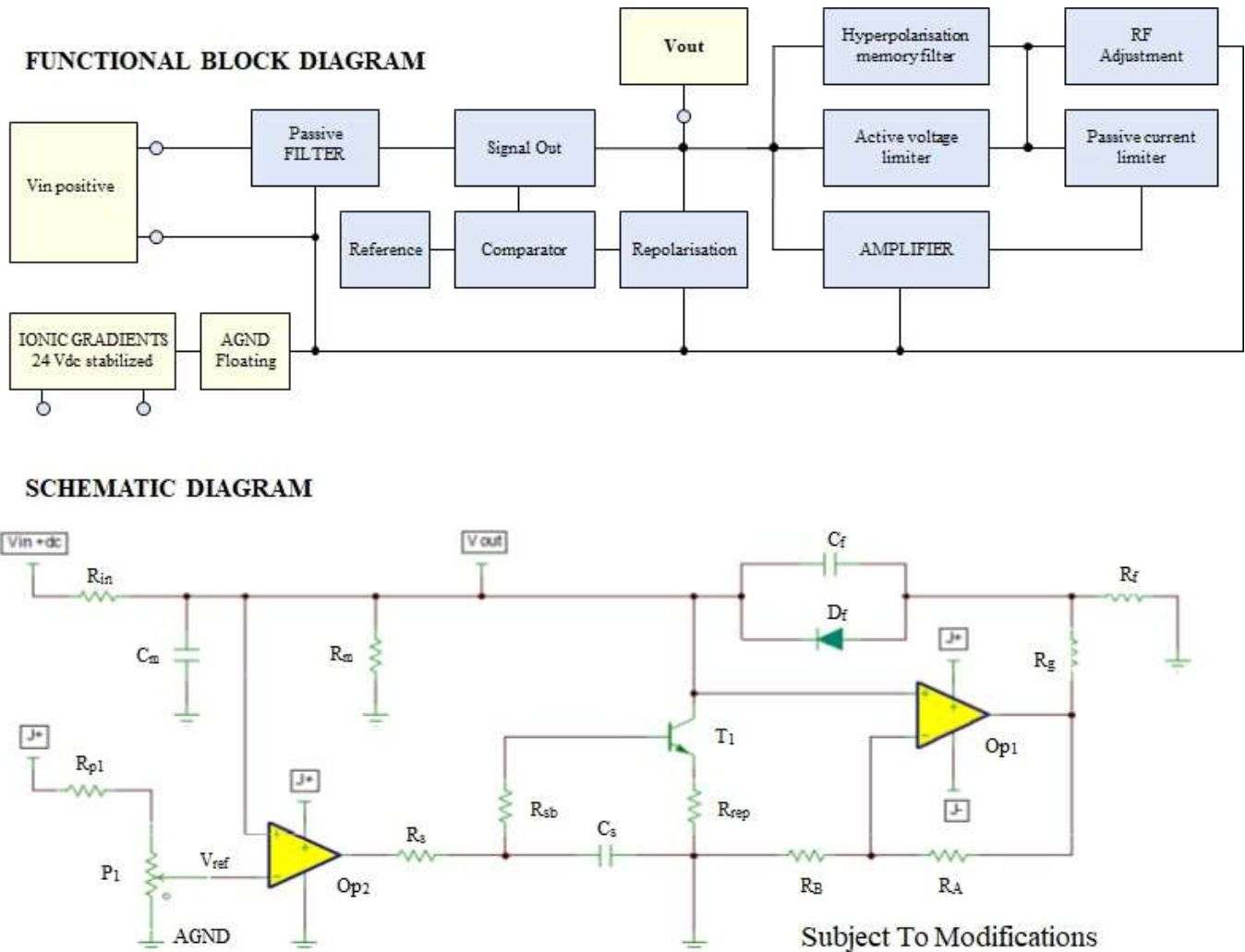No specific voltage-inactivation circuitry is foreseen for

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FUNCTIONAL BLOCK DIAGRAM

SCHEMATIC DIAGRAM

Subject To Modifications

Figure 3. Functional Block Diagram and Simplified Schematics of Neurosimilator's Soma.

this type of current. Accordingly, the repolarization phase of an action potential in Neurosimilator is determined by the rapidly rising discharging current (corresponding to that of potassium) that shunts the charging current coming from the positive feedback of the operational amplifier (corresponding to that of sodium). Since positive feedback is progressively shunted to the ground, diode current fades away before being abruptly annulated once membrane voltage decreases below the threshold value of 0.7 V. Discharging/repolarizing potassium current is generated using a comparator that drives a NPN transistor with a precise delay to sodium currents activation. This off-on/on-off delay is determined by the reference voltage V_{ref} = 3.7 V and RC circuit (R_s=5.6 kΩ and C_s=4.7 μF). Adjusting the values of this delay may allow modification of the neuron transfer function. The capacitor in this positive feedback loop (C_f=10 μF) is responsible for the appearance of the transient hyperpolarization phase at the end of the action potential; during this time, the diode becomes transiently reverse-biased so that the circuit enters into refractoriness. Gain resistance (R_g) in series with C_f is necessary to limit

positive feedback current. The resistance R_f is used for fine tuning of the gain resistance.

The current stimulation of the circuit is achieved by applying a voltage to the pin of an electrode on PCB that has input resistance (R_{in}) of 10 kΩ. The node of Ranvier has essentially the same circuitry as soma albeit with different dimensions and components values (given in Supplementary information). In fact, the node of Ranvier should remain synchronous with a delay determined with respect to the output of the axon

Materials.

The laboratory manipulations require the following equipment:

1. Printed circuit boards representing neuron modules.
2. Laboratory power supply unit delivering 30V DC and a separate power supply adapter as described above.
3. AD-DA interface. We use myDAQ from National Instruments, Inc. that is connected to PC via USB. To facilitate the modification of the cable connections during experiments, MyDAQ is put into an aluminum alloy box and its input and outputs are wired to the panel mount BNC
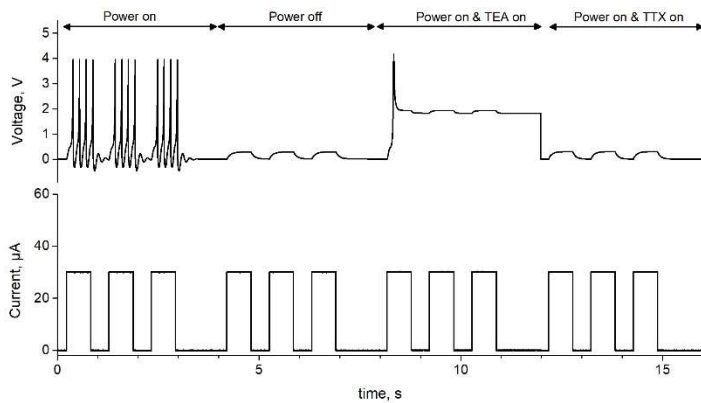
Figure 4. Neurosimilator Behavior in the Presence of Periodic Square Current Input. The circuit is quiescent when the current is zero and spikes during application of 30 µA. Application of TEA depolarizes the circuit and its periodic excitation is blocked. In the absence of power and in the presence of power and TTX, only a passive response is observed.

connectors corresponding to analog output AO0 and two analog inputs, AI0 and AI1. In order to control inputs and outputs of the interface, the WinEDR program is used (Dempster, 1997). Alternatively, a digital oscilloscope may be used to monitor the signals. In this case, a separate electrophysiological stimulator unit is needed. Open source electrophysiological stimulators are readily available (Land et al., 2004; Santos et al., 2019; Cermak et al., 2020).
4. Three coaxial cables with hook connectors (or alligator clips), one regular coaxial cable and a Tee-BNC splitter (BNC male to dual female coaxial connector) or a BNC-hub.

RESULTS
Laboratory session runs for 4 hours.

Experiment 1: Observation of Passive and Active Responses of an Excitable Membrane and Examining the Role of Ionic Conductances in the Generation of Active Response (Action Potentials)
In the absence of stimulation, the neuromorphic circuit is at rest. Current injection into the soma via the pin of stimulation electrode produces a response that can be observed and measured. We propose to apply a periodic square wave signal of 1 Hz, which varies between 0 pA and 30 µA.

In the presence of this stimulus, students perform the following manipulations:
- Observe the response of the circuit in the absence of ion concentration gradients (power box switched OFF);
- Observe the response of the circuit in the presence of ionic concentration gradients (power box switched ON);
- Observe the difference in circuit behavior when you "apply" the tetrodotoxin that blocks potential-dependent sodium channels (TTX-labeled switch on PCB with a red LED). How does the membrane voltage change? What behavior is duplicated in this case? Return the TTX switch to its original position. Under these conditions, the neuromorphic circuit restores its periodic activity.
- Observe the difference in circuit behavior when potential-dependent potassium channels are "blocked" with

tetraethylammonium (TEA-labeled switch on PCB with a red LED). How does the membrane voltage change? (NB. Since Neurosimilator does not contain voltage-inactivation circuitry for Na-currents, the response of the model is similar to the excitation block with high depolarization, described for some neurons). Return the TEA switch to its original position. Under these conditions, the neuromorphic circuit restores its periodic activity.

The expected observations are depicted in Figure 4.

Experiment 2: Determination of the Rheobase.
All remaining manipulations are carried out in the presence of ionic concentration gradients (power box switched ON). When a neuron accumulates enough positive charges (and thus depolarizes), an action potential is triggered. For each neuron, there is a minimal intensity of a current pulse of infinite duration, whose application can depolarize the cell exactly to a threshold potential value, which in turn triggers the action potential. This current intensity is, by definition, the rheobase. Accordingly, students are asked to subject the neuron to injections of direct current (thus of *quasi infinite* duration). Prior to current manipulations, the circuit should be brought into the resting state - the periodic square protocol application from the previous experiment should be stopped and the applied DC current value must read zero. Students should find the minimum DC current pulse intensity (between 10 µA and 30 µA, to the nearest 0.1 µA) that triggers an action potential. Different current pulses are ALWAYS tested on the circuit put back to the resting state (i.e., 0 µA applied between each new current value). After students determine the rheobase in the case of a neuron with a myelinated axon, they are asked to perform the measurements of rheobase in the case where the axon connected to the neuron is replaced by the the demyelinated axon.

The results obtained by students demonstrate to them that the rheobase of their neuron model with demyelinated axon is slightly higher than that with myelinated axon. They have to explain if this observation is coherent with the modification of passive properties of Neurosimilator, when two types of axons are connected separately to the soma. Obviously, a demyelinated axon provides more current leaks to the ground in parallel to the current leaks in the soma, although these axonal leaks remain minor.

Experiment 3. Passive Signal Propagation in the Axon
It is commonly accepted that passive axons (i.e., the regions of axons that do not contain voltage-dependent channels) can be assimilated to a poorly insulated electric cable having internal resistance that increases with the length and a leak resistance that decreases with the length considered. The space constant λ is defined as the length of the nerve fiber such that the longitudinal resistance is equal to the membrane leak resistance. The first manipulation part is done in configuration with the soma connected to the dendrite and to the demyelinated axon followed by the node of Ranvier. Periodic activity of the neuromorphic circuit is generated by application of a 20 µA DC current into the soma via stimulation electrode. This activity is recorded for
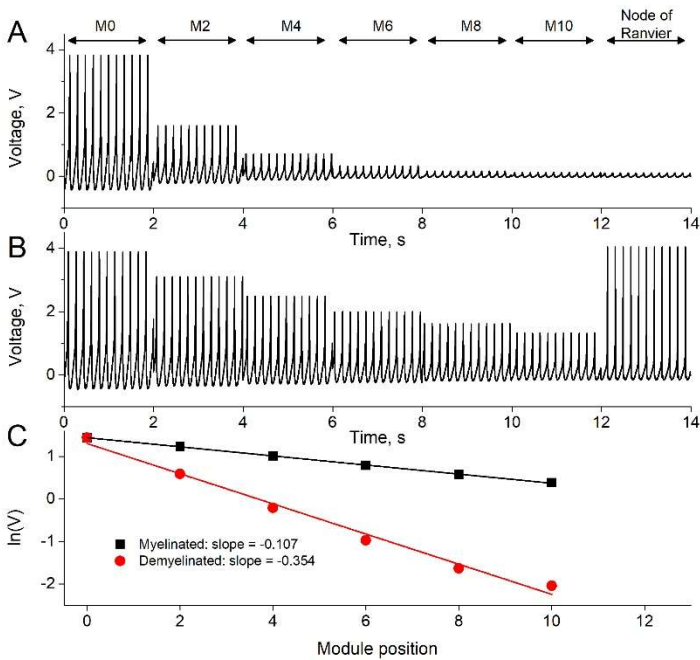
Figure 5. Measurement of Passive Signal Propagation in Demyelinated (A) and Myelinated (B) Axon and Analysis of Obtained Data (C). Theoretical slopes calculated using equation 1 and values of R_a and R_{axo} are equal to -0.346 and -0.109 for demyelinated and myelinated axon, respectively.

1 second consecutively from the pins of modules M0, M2,M4, M6, M8 and M10 of the axon as well as in the node of Ranvier by moving the red hook of the "recording" cable. Peak-to-peak signal amplitude associated with the action potentials is measured off-line for each recording in the data file. It can be necessary to adjust the time display scale for more precise measurements and to use the scan bar in order to navigate through the file. These manipulations are then repeated with the myelinated axon replacing demyelinated.

The results of these experiments should be presented in graphical form by plotting the amplitude (in V) vs. position of the module, as well as by plotting the logarithm of the amplitude vs. position of the module (Figure 5). In latter case, the slope of the curve is calculated using MS Excel. Students are asked to relate the two calculated slopes with the electrical properties of the two axons used. Clearly, a logarithm of Equation 1 gives a linear expression with a slope coefficient equal to the negative and inversed value of the space constant. Expectedly, there is no electrical activity detected in the node of Ranvier if the demyelinated axon is used, since the signal amplitude is strongly attenuated.

Experiment 4. Refractory Period.
Generally speaking, a refractory period in case of neurons corresponds to a time interval following the end of an action potential during which the suprathreshold stimulation is incapable to trigger another action potential. Students observe and record the response of the soma to the periodic "Refractory" protocol applied directly to the soma via the stimulation electrode. This preprogrammed stimulation corresponds to a train of two supra-threshold current pulses
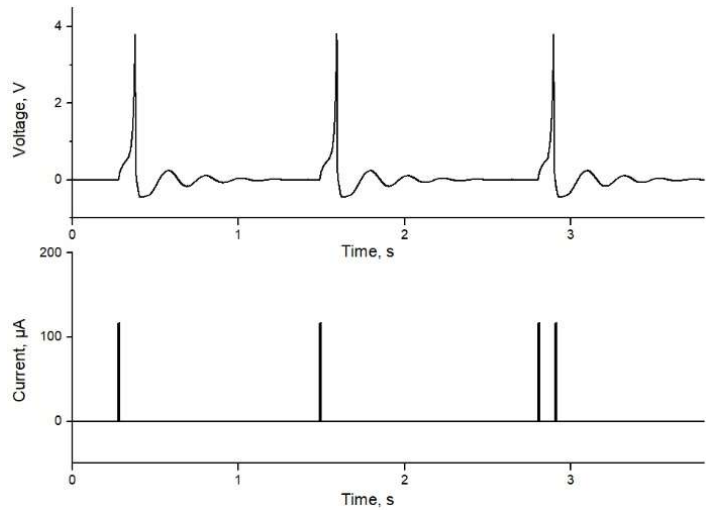

Figure 6. Observation of Refractory Period. Second suprathreshold current pulse applied briefly after the first one does not produce second action potential.
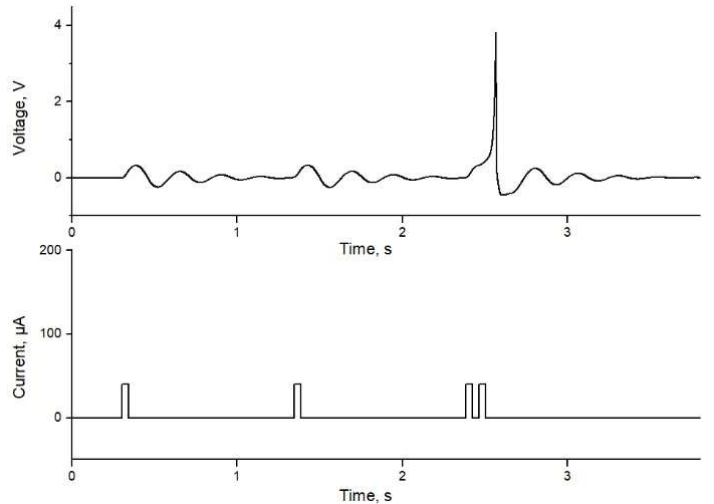

Figure 7. Observation of Temporal Summation of Dendritic Currents. Second subthreshold current pulse applied briefly after the first one produces an action potential.

of identical amplitude and duration with large delay between them, followed by a train of two same current pulses with short delay between them (Figure 6). With an adjustable electrophysiological stimulator, students would be able to measure the exact duration of the refractory period by reiteration of the application of two identical supra-threshold current pulses with variable delays between them. Accordingly, maximal delay not producing a second excitation equals the relative refractory period under these stimulation conditions (amplitude and duration of supra-threshold current pulses). It corresponds roughly to the time period of after-hyperpolarization.

Experiment 5. Temporal Summation of Dendritic Currents.
Usually, most of neuronal information comes to the soma of a neuron via its dendrites. Following this rule in the

experiments, we connect the stimulator cable to the D1 module of the dendrite. Students observe and record the response of the soma to the periodic "Summation" protocol. This preprogrammed stimulation corresponds to a train of two sub-threshold current pulses of identical amplitude and duration with large delay between them, followed by a train of two same current pulses with short delay between them. Students measure the delays between the two pulses for both cases and try to relate these delays to the passive properties of the soma in order to explain the soma behavior's striking difference in two cases (Figure 7).

Experiment 6. Spatial Summation of Constant Dendritic Currents.

For these experiments, one needs to simultaneously apply two identical direct current stimulations to different dendrite branches. To do so, students connect another coaxial cable with hooks to the same output of the simulator/interface using Tee-BNC splitter. Red hooks of both cables are then connected to the end of two dendrites (modules D3 and D3'). A sub-threshold DC current of 15 µA is applied (hence, 7.5 µA in each cable). Students move the red hooks of cables (one at the time) on the modules closer to the soma in steps of one module at a time, in order to find the farthest spatial configuration that triggers action potentials. Several configurations are usually possible, in which the removal of one of the cables stops immediately spiking activity. In real neurons, however, dendrites never receive constant synaptic currents and thus purely spatial summation does not occur. Instead, dendrites and soma perform spatio-temporal summation, the experimental protocol for which is described below for advanced students.

Experiment 7. Strength-Duration Relationship and Determination of Chronaxie.

If one applies current pulses of finite and variable duration to the soma, it can be seen that the minimal threshold intensity of the current pulse decreases as the duration of the pulse increases, with a global minimal intensity being the rheobase. To put it differently, the threshold of the action potentials is reached more rapidly as the current intensity of the pulse increases. Students are asked to construct the strength-duration curve by plotting the intensity value of the minimal trigger current pulse as a function of its applied duration. This curve demonstrates a parameter of great interest in physiology – the chronaxie, which is the minimum duration of a rectangular step of current with double intensity of the rheobase that triggers an action potential. The importance of chronaxie is that it varies very little with the experimental conditions and that it depends only on passive properties of the cell (Lapicque, 1907). This manipulation is performed using only the soma, i.e., all other modules should be disconnected.

In order to perform this experiment, the stimulator should allow adjusting the amplitude and the duration of the pulse. The most flexible option is to use a software-driven DA-interface. To speed up the manipulation, the exact durations of the current pulses are communicated to students. Students then iterate the pulse application to find the
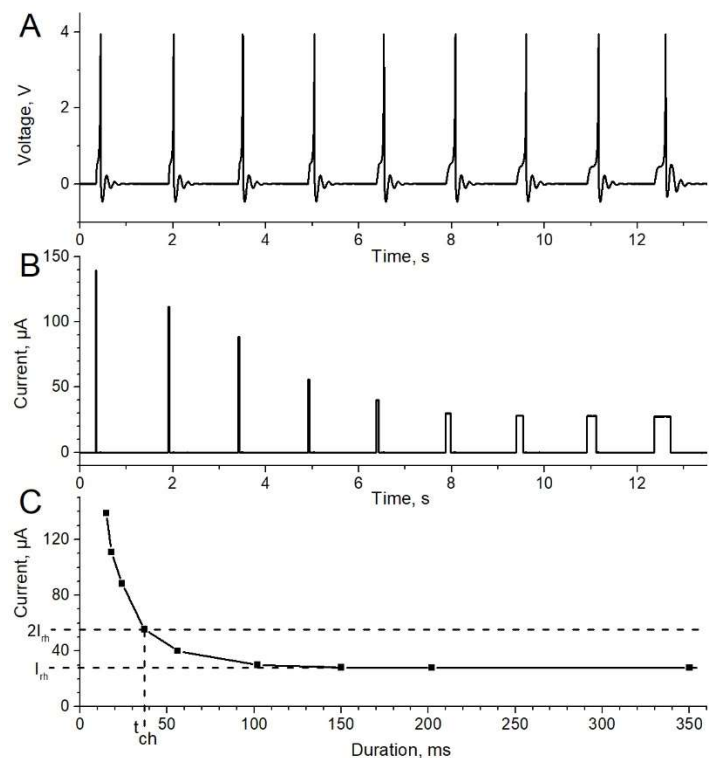

Figure 8. Demonstration of Strength-Duration Relationship (Weiss-Lapicque Law). Experimentally obtained value of chronaxie in this experiment was 37 ms, which is close to the theoretical value of 36.7 ms.

minimum trigger current (within 1 µA) for each duration. In order to find the exact value of the chronaxie, students look for the minimum pulse duration (to the nearest 1 ms) with the current amplitude equal to the double of the rheobase measured. The obtained value of the chronaxie should be related to the passive properties of the circuit by students in their lab report. Analysis of the circuit suggests that R_m is in parallel with R_{in}, so the equivalent membrane resistance of 10 kΩ ‖ 1 MΩ is equal to 9.9 kΩ. It is more difficult to estimate equivalent membrane capacitance of the circuit; fitting the values of discharge curve in passive conditions (Figure 4) gives only C_m value, because positive feedback capacitor C_f is out of service, if operational amplifier is not powered. On the other hand, C_m and C_f can be considered to be in series with R_g between the ground and the point in the circuit having maximal voltage, which is at the output of the operational amplifier. In addition, the values of employed surface-mount capacitors have 10% tolerance. A good estimation of the equivalent membrane capacitance, which is in agreement with experimental measurements, was fixed to be 5.35 µF. These figures ($R_{m,eq}$=9.9 kΩ and $C_{m,eq}$=5.35 µF) are communicated to students; which allows them to calculate the theoretical chronaxie value of 36.7 ms. Expected observations and analysis are depicted in the Figure 8, which shows good agreement between experimental and theoretical values of chronaxie.

Experiment 8. Neural Coding by Firing Rate and the Effect of Cell Size on Excitability

This coding model assumes that most (if not all) of the

neuronal stimulus information is contained in the discharge (spiking) frequency of the neurons. The neuronal frequency coding model, therefore, states that the frequency of action potentials is proportional to the intensity of the stimulus to be transmitted. The proposed experiments reproduce the pioneering work of Hartline and coworkers, in which they measured changes in electrical activity of the optic nerve of the Lumulus crab produced by variable illumination (Hartline at al., 1956).

In order to proceed with the experiment, the neuromorphic circuit is brought to resting conditions: no external stimulation is needed and the stimulation cable may be disconnected. In order to engage the photoreceptor, switches PHR and S1 on PCB should be turned to the upper position. Under these conditions and in the presence of ambient light in the classroom, the circuit produces weak electrical activity. This activity is abolished if the photoreceptor is covered with a piece of cardboard. Alternatively, students may progressively approach a flash of their smartphones to the photoreceptor to realize that the activity increases with rising light intensity.

In the second experiment, students adjust the light to observe very minimal firing activity of the circuit by partially covering a photoreceptor. Then, they carefully disconnect the dendrite, axon, and node of Ranvier. Students observe the increase in spiking activity and then try to explain the effect of the module's removal on the firing rate. The expected explanation is that the capacitances of dendrites and axon are both in parallel with the soma capacitance, so that the removal of modules decreases overall membrane capacitance, while all voltage-dependent currents are still generated in the soma only. As these same currents need to charge smaller capacitance, the rate of spiking activity is accelerated.

Experiment 9. Velocity of Passive Propagation of Action Potential Signal in the Axon

First, students apply a current pulse of 20 µA amplitude and 50 ms duration with a 500 ms delay to the pin of the stimulation electrode, then simultaneously record 1 second of activity in the soma. Next, they move the red hook of the recording electrode from the soma to the M10 module of the axon and repeat the recording (having restarted the stimulation pulse protocol). In order to measure the signal propagation duration between the soma and M10, students measure the delay between the start of the current pulse and the peak of the action potential in the soma, and then the delay between the start of the current pulse and the peak of the signal recorded at module 10. Time measurements should be done with an accuracy of 0.1 ms. The propagation time of the signal is simply the difference between the two measured delays. Alternatively, both signals (from the soma and from M10 module) can be recorded simultaneously on two interface channels (AI0 and AI1) and the delay of signal propagation can be measured directly between the corresponding peaks. Students repeat this experiment with myelinated and demyelinated axons. They are told that propagation time in case of myelinated axon is below 1 ms. Given that there are 10 modules between soma and M10,

the signal propagation velocity (in terms of modules per second) can be calculated for the two types of axons. Again, students should relate slower propagation of the signal in demyelinated axon with its higher membrane capacitance compared to myelinated axon.

Experiments for Advanced Students

Students may cooperate by using their neuromorphic circuits to study spatio-temporal summation of neuronal signals. To this end, soma and dendrites of one Neurosimilator should be used as a summator neuron of two other Neurosimilators as interneurons. These two interneurons should be stimulated to generate tonic spiking activity, which is then fed to different dendrite branches of summator neuron. For this type of experiment, additional non-coaxial cables with hooks are required to connect the somas of interneurons to the dendrites of summator neuron and to interconnect the reference points (grounds) of all the neurons. Depending on the position of the connectors on the dendrites of the summator neuron, various periodic spiking patterns may be observed in the summator soma.

Simple neural network can be constructed and studied with bigger number of cooperating Neurosimilators. One good example is a memory loop, in which Neurosimilators form a series of at least 5 circuits each comprising dendrite, soma, myelinated axon and node of Ranvier. The node of Ranvier of the fifth neuron is connected to the dendrite of the first neuron. In this configuration, single and very brief supra-threshold stimulation of one of the neurons generates action potential that will circle in this loop without any further additional stimulation.

The Neurosimilator is capable of reproducing the following spiking patterns (according to Izhikevich, 2004): tonic spiking and subthreshold oscillations (experiment 2), tonic bursting (using external PCB board), accommodation, refractory period (experiment 4), summation (experiment 5), Class I excitation (experiment 8), spike latency, threshold variability. Observation of these patterns requires only a specific stimulation protocol. With additional circuits, it may show phasic spiking, phasic bursting, Class II excitation and bi-stability. Teachers and advanced students interested in learning more on non-linear dynamics mechanisms behind the oscillatory behavior of Neurosimilator may refer to our recent publication (Shlyonsky et al., 2024).

Assessment of Students' Feedback

We have performed an anonymous survey of students' perception of the utility of the majority of the proposed lab activities. Analysis of students' feedback shown in Figure 9 demonstrates that students remained consistent in their overall positive appreciation of this laboratory activity. The majority of the students agreed that the theory of the course was well depicted during manipulations, which helped them understanding complicated notions. They also agreed that this lab activity represents a challenge and thus good preparation for the lab session is mandatory. This may partially explain why the students estimated that more time is needed to complete the lab. Finally, although they recommend the lab to other students, the majority of the
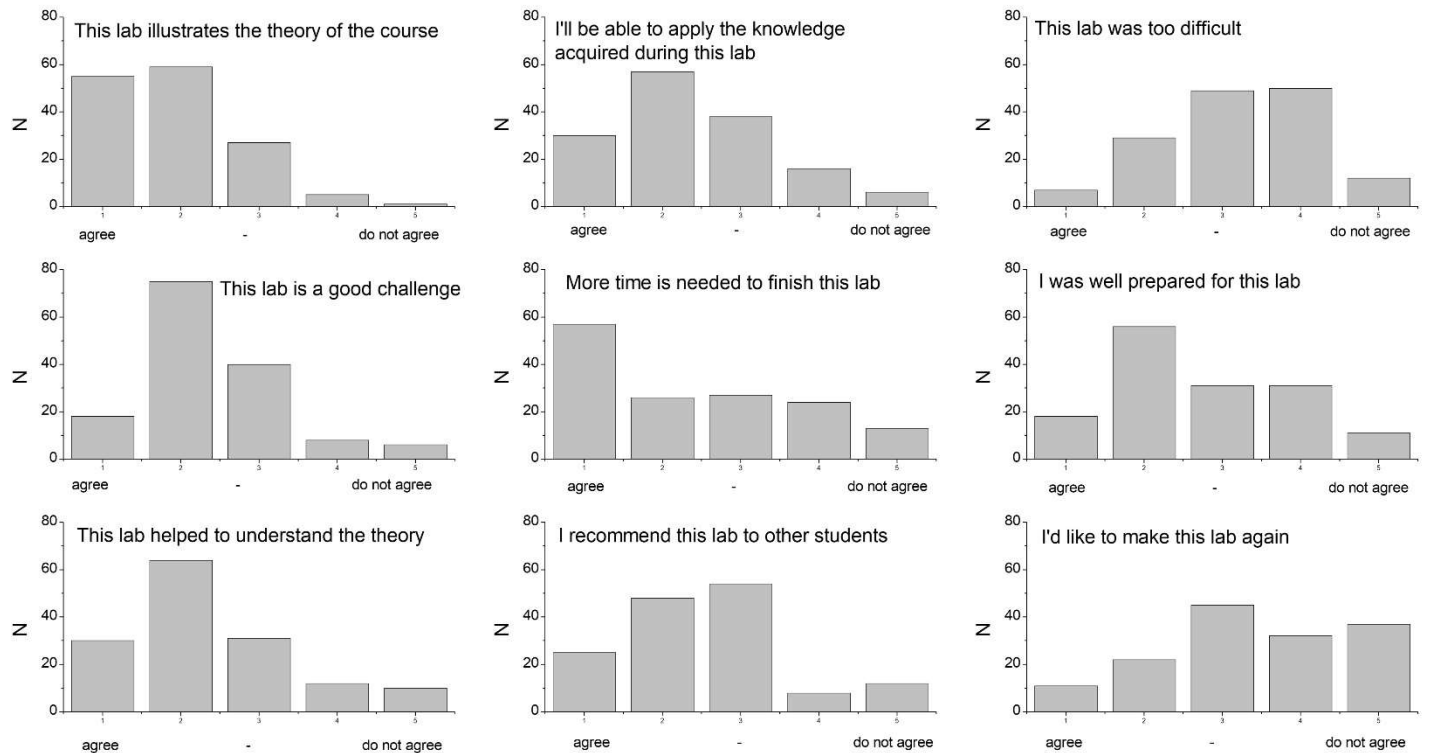
Figure 9. Students Feedback Analysis.

students did not intend to perform the lab again, probably because of lack of time in their charged premedical curriculum or because they've already achieved the learning objectives.

DISCUSSION

Enriching the neuroscience class with hands-on experience may be challenging, however many possibilities do exist. For example, simple RC-circuits are broadly used and proved excellent means to study passive membrane properties (Dabrowski et al., 2013). On the other hand, working with living objects in a neurophysiology classroom that does not require animal ethical committee approval is still possible. The Spiker Box amplifier proposed by the Backyard Brains company comes with a number of carefully described electrophysiological/biophysical experiments on cockroaches, crickets, earthworms and even plants such as Venus flytrap and mimosa (Marzullo and Gage, 2012). These experiments permit observation of action potentials and their pharmacological modulation, neural coding by firing rate and temporal summation and allow action potential's conduction velocity measurements (Kladt et al., 2010; Dagda et al., 2013; Shannon et al., 2014; Nguyen et al., 2017). More sophisticated and didactic biophysical experiments may be conducted with hardware models of neurons. The accessible ones include microcontroller- and FPGA-based models such as NeuroBytes (Petto et al., 2017; Burdo, 2018), Spikeling (Baden at al., 2018) and those developed at Cornell University (Land, 2014; Land,

2016a; Land, 2016b). The advantage of programmable hardware neuron models is that they are tiny and that with the same materials any available neuronal model can be computed, digital-to-analog converted and observed at the output. This hardware reproduces a lot (if not all) of neuron behavior patterns. Setting up these models, however, requires intermediate or even advanced coding competences. Fully analog hardware neuron models developed for undergraduate teaching exist as well (Koch and Brunner 1988; Rutherford et al., 2020). These are real-time models as opposed to microcontroller-based, they are very accurate in reproducing excitability of neurons, but still bulky and complex (even if built with modern integrated circuits) and require a number of fine tunings. The model proposed in our work combines simplicity and versatility and as such constitutes a good addition to the panel of open-source projects aiming to aid the neuroscience/biophysics teaching environment.

Conclusions

By doing the proposed hands-on experiments using neuromorphic circuits, students have the opportunity to observe in practice complicated notions from biophysics/neurophysiology curriculum by doing inquiry-based experiments. According to student survey results, these manipulations helped students to understand important notions of the course. Thus, our neuromorphic circuit could be a valuable tool for biophysics and neurosciences courses in other universities as well.

Supplementary Easy EDA Files:

https://github.com/Shlyonsky/Neurosimilator

PCB_AXON-demyelinated.json
PCB_AXON-myelinated.json
PCB_DENDRITES.json
PCB_RANVIER'S NODE.json
PCB_SOMA.json

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