

## TECHNICAL PAPER

### 3D Printing Neuron Equivalent Circuits: An Undergraduate Laboratory Exercise

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The electrical equivalent circuit for a neuron is composed of common electrical components in a configuration that replicates the passive electrical properties and behaviors of the neural membrane. It is a powerful tool used to derive such fundamental neurophysiological equations as the Hodgkin-Huxley equations, and it is also the basis for well-known exercises that help students to model the passive (Ohmic) properties of the neuronal membrane. Unfortunately, as these exercises require basic knowledge of electronics, they are generally not physically conducted in biomedical courses, but remain merely conceptual exercises in a book or simulations on a computer. In such manifestations, they lack the “hands-on” appeal for students and teachers afforded by laboratory experimentations. Here, we propose a new approach to these experiments in

which a desktop 3D printer and conductive paint are used to build the circuit and the popular programmable microcontroller, the Arduino UNO, is used as a graphical oscilloscope when connected to a standard computer. This set-up has the advantage of being very easy to build and less clumsy than a circuit in a prototyping board or connected with alligator clips, with the added benefit of being conveniently portable for classroom demonstrations. Most importantly, this method allows the monitoring of real-time changes in the current flowing through the circuit by means of a graphical display (by way of the Arduino) at a fraction of the cost of commercially available oscilloscopes.

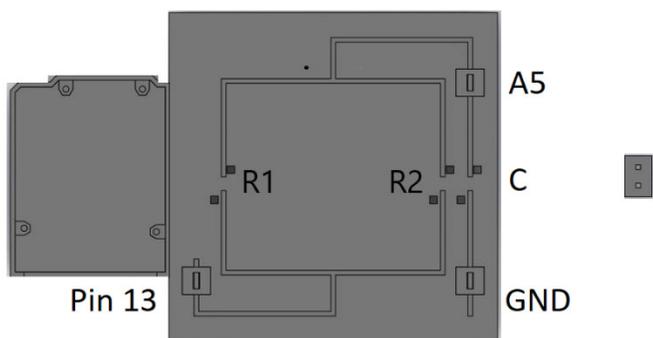
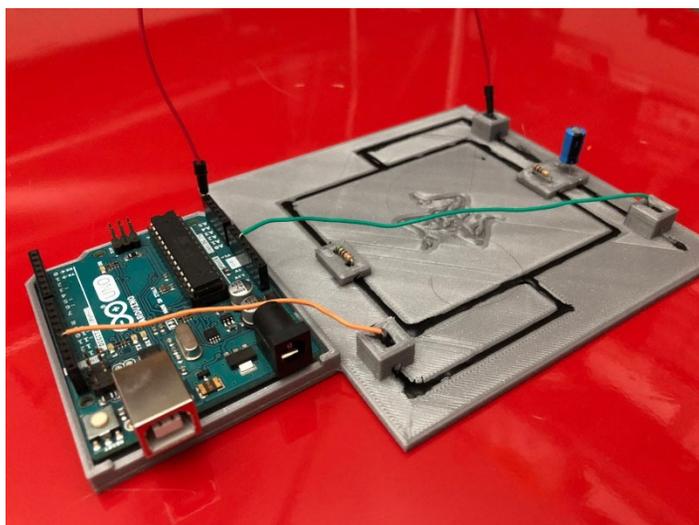
*Key words: electrical equivalent circuit; neuron electrical model; Arduino UNO; 3D printing; oscilloscope; DIY*

Learning the basic laws and properties that govern the neuron’s membrane physiology and electrical behavior is a crucial intended learning outcome in the neurosciences. The origin of the resting membrane potential and its relationship to Nernst equilibrium potentials (Goldman et al., 1943; Reinmuth 1957) is relatively simple for biomedical students to learn, as they generally have sufficient background in biochemistry and biophysics. Other properties, however, like the *membrane time constant*, can be more difficult for undergraduate science students to grasp. The membrane time constant  $\tau$  describes the time it takes for the membrane potential to change by about 63% of its steady-state value, in response to a current input (Kandel et al., 2013). This property has relevant consequences on neural physiology, in particular in the way post-synaptic potentials summate and non-regenerative signals rise and decay over time. The reason for the lack of accessibility of these fundamental concepts is that while most biomedical students have a solid foundation in chemistry when they begin their neuroscience studies, very few have had more than a cursory introduction to the field of electronics, or may never see it at all during their physics course. This problem is compounded further when a large number of neuroscience students are introduced to the field through psychology, computer science or the humanities, in which case they may be playing catch-up in the basic sciences as their appetite for knowledge of the neural substrates of behavior grows and develops.

The so-called ‘equivalent circuit’ (EC) was first proposed by Hodgkin and Huxley (1952). According to this model, an RC circuit (a simple electrical circuit containing a resistor

and a capacitor in a parallel configuration) can be used to simulate the main passive electrical properties of neuronal membranes. The ion channels are modeled as resistances (Fletcher et al., 2014). While physiologists normally discuss ion channels in terms of conductances (the inverse of resistance), the sum total of the membrane’s conductance is most often measured in Ohms (input resistance). As conductance ( $G$ ) =  $1 / \text{resistance (R)}$ , a Siemen of conductance is equal to  $1 / \text{Ohm}$ . When  $N$  ion channels (where  $N$  is a positive integer) are placed in parallel the total conductance of the membrane is  $N$  times the conductance of a single channel. Similarly, when  $N$  resistors of the same magnitude are connected in parallel, the inverse of the total resistance of the circuit ( $1 / R_T$ ) is equal to  $N/R$  (where  $R$  represents a single resistor in Ohms).

The concentration gradients across the membrane are typically represented as *batteries* (for examples, see Crisp, 2019; Dabrowski et al., 2013; Robinson et al., 2011; Wyttenback et al., 1997). This can be confusing for students to understand, as each electrochemical gradient in a nerve cell is often depicted as a different battery (a  $-77$  mV battery for potassium, in parallel with a  $+45$  mV battery for sodium, etc). The membrane capacitance is represented by a *capacitor*, an electrical component whose structure is characterized by two layers of conductive material, called *armatures* (to represent the cytoplasm and the extracellular fluid), separated by a dielectric insulating medium, corresponding to the phospholipid bilayer, which forces the ions to flow through the channels (Wang et al., 1989). Inside the dielectric, an electric field is generated following the external application of a voltage on the armatures. The unit



*Figure 1:* The 3D printed components. The main board is divided into a smaller section (left) which holds the Arduino microcontroller and a larger working space (right) in which resistors (R1 and R2) and capacitors (C) can be attached and detached easily. There are also three deep square wells in which wires will be attached that can plug into the pin connectors on the Arduino. These wells are labeled in the diagram according to the pin connector on the Arduino that they connect to: analog input pin A5, digital output pin 13 and any of the three ground (GND) pins. The second, much smaller printed piece (far right) is printed (at least) in quadruplicate, and contains two holes through which a resistor or a capacitor can be attached. Attaching resistors and capacitors is thus as simple as plugging the smaller printed part holder into the appropriate spot on the working space, where it fits snug in a complementary fashion, not unlike Lego building blocks.

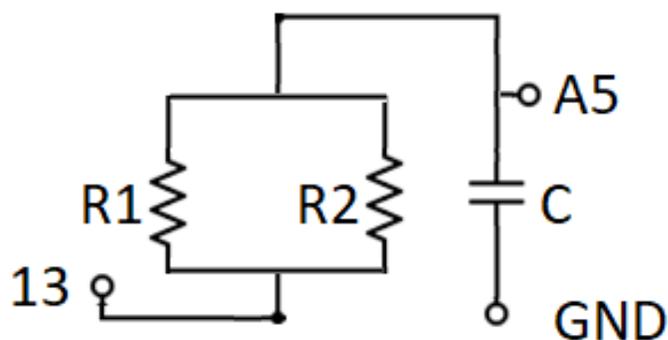
of measure of capacitance is the farad.

The current flowing in the circuit can be visualized by means of a voltmeter or an oscilloscope, which graphically plots variation of current over time. Recently, an undergraduate laboratory exercise was proposed by Dabrowski et al. (2013), in which a very effective step-by-step guide helps students to build their own EC, using alligator clips instead of traditional wires, thus avoiding soldering and also allowing students to play with the circuit by subtracting or adding circuit components on the fly and observing the effects. Using this model system, students can measure membrane time constants, observe how adding or removing ion channels (by adding and removing resistors) affects membrane potential and time constant, and view the contribution of capacitance to the time constant

of the membrane.

Here, we introduce an alternative laboratory exercise that introduces students to the same concepts while engaging them in work with emerging STEM tools. First, we propose the use of a cheap (\$20) Arduino microcontroller to serve both as a square wave stimulator and (in conjunction with a computer monitor) as an oscilloscope. Second, we propose the use of a 3D printed circuit to reduce the number of connections a student needs to manage while manipulating the circuit. The 3D printing could be done in advance without student involvement, generating a set of conveniently stored, ready-to-use circuit set-ups that can be set up in lab quickly. Or, 3D printing can be done in cooperation with the students, either in or out of lab, potentially introducing students to a new and widely usable STEM skill set. In the last few years new cost-effective and affordable technologies in DIY manufacturing, like 3D printing, changed the way people make their own devices, lab equipment included (Baden et al., 2015). The same is true of the Arduino programming, in that the Arduinos can be preprogrammed (using the code included below), or the students could be encouraged to program their own microcontrollers (or at least modify the code themselves, to change parameters such as the stimulation parameters). The availability of programmable open-source microcontrollers like the Arduino and Raspberry Pi boards allow people to build flexible and effective smart objects like robots or home automation devices. The Arduino board also has been proved to be precise and accurate, and thus suitable for science experiments (D'Ausilio et al., 2011; Schubert et al., 2013).

Here, we propose a new version of the EC, in which the basic circuit is made by 3D-printing troughs filled with electro-conductive paint and the electronic components are embedded in 3D-printed holders that can be removed or added on the fly to the circuit. Moreover, an Arduino Uno



*Figure 2:* Circuit diagram of the printed project shown in the Figure 1. Note that the capacitance is in series with the resistance in this circuit, not parallel as is envisioned in the cell membrane. This provides electrical isolation between the input and output pins of the Arduino. The time constant is the product of resistance and capacitance whether or not they are in parallel or in series. According to Thévenin's Theorem, the parallel RC configuration of the membrane is a linear circuit that can be reduced to a single voltage source and a single resistance in series with a load, which is in this case the capacitor across which the voltage is read by the Arduino.

connected to a computer works as both stimulator and oscilloscope, increasing the visual teaching efficacy by showing current changes over time as compared to a simple voltmeter. If a 3D printer and a PC are already available, the total estimated cost of the materials used in the experiment is about €20-22 (\$22-25 USD).

## MATERIALS AND METHODS

1. A desktop 3D printer: We successfully used a cheap FDM model, but in principle any 3D printer with a printing bed of at least 20 x 15 x 3 cm should work fine. For example, we also used a TAZ 6 in conjunction with the CURA (3.6.3) software package.
2. An electro-conductive paint or any other electro-conductive material. We successfully used Bare Conductive (<https://www.bareconductive.com>), which has a resistance of about 55 ohms/square at 50 microns layer thickness. Alternatively, we have also had success using a DIY recipe (described below).
3. An Arduino Uno microcontroller (<http://arduino.cc>) with three jumper wires, preferably different colors.
4. Two resistors (1 M $\Omega$ , labeled brown-black-green).
5. Two ceramic disk capacitors (100 nF, labeled "104", and 1  $\mu$ F, labeled "105").
6. One computer (PC, Mac or Linux) with an internet connection to download the free software from Arduino.cc.

### Step by Step Procedure

Step 1: Download our printable .stl model from <https://www.thingiverse.com/thing:3189826><sup>1</sup>. If you are new to 3D printing, an .stl file format (abbreviation for sterolithography) is the format produced by designers using CAD or related software packages. It contains the specifications for the surface geometry of the object to be printed.

Step 2: 3D print the base and four component holders (these are the little rectangular pieces shown in Figure 1) at 0.3 mm resolution, 30% infill (noncritical since it is mainly flat). Use skirts for the base (should be an option in most software packages). Both PLA and ABS can be used as printing material.

Step 3: Fill the troughs and wells of the base with the electro-conductive paint. Let dry and fill again to be sure all holes are filled in; there should be no gaps. Once completely dry, test for electrical continuity using a volt meter. Note that opened electro-conductive paint has a limited shelf-life and is prone to drying out. Fresh paint is easier to work with. A cheap, DIY electro-conductive paint can also be made using a mixture of approximately equal proportions (by mass) of graphite powder (we used AGS Extra Fine Graphite Dry Lubricant, sold in hardware stores for lubricating locks) and white schoolroom glue (such as Elmers), blended together very thoroughly and used fresh. If necessary, this can be diluted down to a ratio of 1:3 graphite to glue (parts mass).

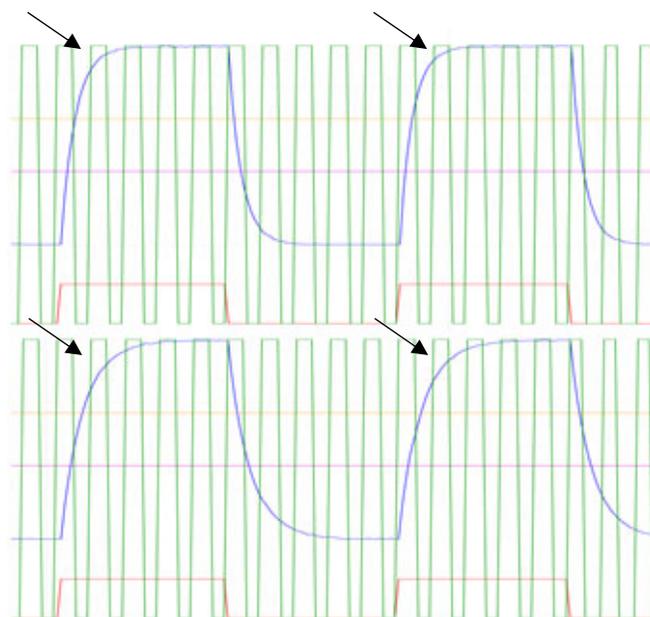


Figure 3. Effect of “ion channels” on membrane voltage rise time. In the upper panel, the circuit consists of a 100 M $\Omega$  resistor at R1, a 100 nF capacitor at C and R2 is open. In the lower trace, there is also a 100 M $\Omega$  resistor at R2. This simulates a doubling of the number of open ion channels in the membrane, and it is evident from the charging curves that the time constant is approximately twice as long. Note that there is stray resistance from the electroconductive paint throughout the circuit that may make the measured time constants significantly longer than would be predicted from the resistor and capacitor alone. These exercises are meant to visually relate the time constant to resistance and capacitance, as a supplement to simulations and calculations traditionally done in neurobiology classes.

It is wise to paint the wire leads in place in the wells, as it will not be easy to insert a lead into dry paint.

Step 4: Insert the leads of the components (resistors and capacitor) into the holes of the component holder and bend them laterally.

Step 5: Fill the gap of the component holders with the electro-conductive paint. Let dry and fill again to be sure all holes are filled in. The paint on the underside of the component holder will need to make an electrically continuous pressure contact (“cold joint”) with the paint filling the troughs on the base when the pegs of the component holders are inserted into their corresponding plugs on the base.

Step 6: Download the latest Arduino IDE (<https://www.arduino.cc/en/Main/Software>) and copy-and-paste or type the script found in the Appendix to the editor window and upload it in the Arduino using the right-arrow button in the top left corner of the GUI (further documentation on uploading sketches at <https://www.arduino.cc/en/Guide/ArduinoUno#toc6>);

<sup>1</sup> Please note that we designed the circuit but the embedded Arduino Uno holder is a modified version of Arduino Uno Holder/Case by superwoodle

Step 7: Screw the Arduino UNO to the printed holder using standard screws and connect the leads as follows: the lead from the well-marked GND to any of the 3 GND pins on the Arduino; the lead from the well labeled A5 in Figure 1 to analog input pin A5 on the Arduino; and the lead from the pin 13 in Figure 1a (top) to the digital output pin 13 on the Arduino.

Step 8: Connect the components to each place, according Figure 1a. Pin 13 from the Arduino board will attach to the well closest to the Arduino. Any ground pin on the Arduino can be attached to the well in the bottom right. Pin A5 on the Arduino board will attach to the well in the top right corner. A schematic illustrating the printed circuit is shown below.

Step 9: Connect the Arduino UNO to the PC and start the Serial Plotter (Tools menu) in the Arduino IDE.

Note that if you are using the Arduino connected to a laptop that is plugged into the wall with a trickle charger, you *may* see noise on the Arduino software's Serial Plotter window that could render the experimental results difficult to interpret. See Appendix A: Troubleshooting, for an example.

## STUDENT EXERCISES

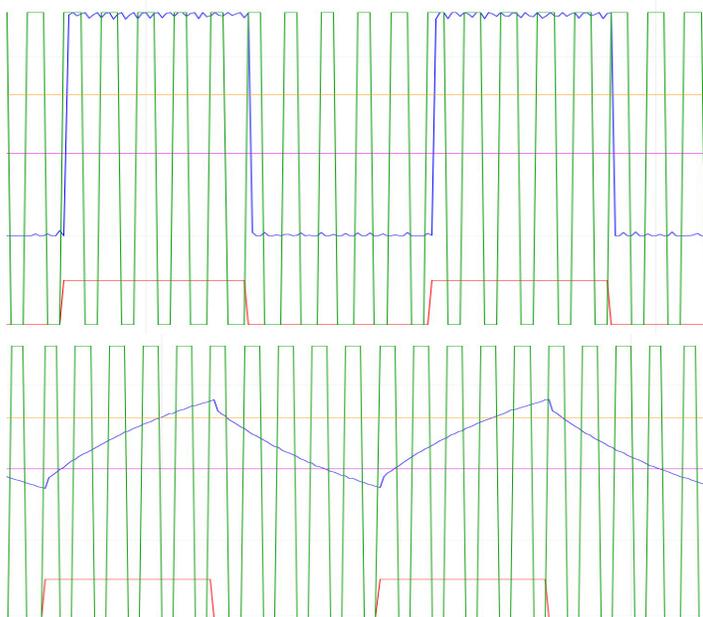
- The membrane time constant is the product of the total membrane resistance and the membrane capacitance. Observe the voltage trace with a 100 nF capacitor at the C position of the base and 100 M $\Omega$  resistors at R1 and R2. Note that although a 5 volt stimulus wave is being applied to the circuit "membrane", the voltage does not change instantaneously (Figure 3A). It takes time to change the voltage by 5 volts. The time it takes for the membrane potential to change by about 2/3rds of the maximum value (about 3.3 V, the orange horizontal line) is the time constant  $\tau$ . Membrane time constant can also be measured starting at 5 volts and noting the time required for the voltage to drop by two thirds (the purple horizontal line). The voltage reaches its peak after a duration of about 5 times  $\tau$ . The vertical green lines represent 100 ms divisions that can be used for measuring elapsed time (as the x-axis in the Serial Plotter window is in iterations rather than units of time). It can be very difficult to measure a moving trace, but connecting a jumper wire between digital pin 1 and either 3.3 or 5.0 volts will pause the screen while you make your estimate the time constant. Calculate the time constant you measure and compare your observations to your calculations.
- Remove the capacitor from position C, so that the circuit now consists of only a resistor. How does this affect the amount of time it takes for the voltage to change in the circuit? See Figure 4A.
- A capacitor is really just two large plates with an insulator between them that can store charge and discharge it later. It takes time for charges to fill a capacitor, and time for it to discharge later. The cell membrane can be thought of as two large surfaces along which ions can be "stored", with an insulator between them (the fatty acid tails of the phospholipids) that is impermeable to ions. While membrane capacitance per unit area tends to be constant between cells, larger cells have greater surface area and therefore greater net capacitance. Simulate a larger cell by exchanging the 100 nF capacitor (#104) for a 1  $\mu$ F capacitor (#105). How does the increased "membrane surface area" affect the membrane time constant? Measure the new time constant and compare it with your calculations. (See Figure 4B).
- Replace the 100 nF (#104) capacitor at the C position. Because parallel resistances sum according to the rule  $R_{total}^{-1} = R_1^{-1} + R_2^{-1} + \dots + R_N^{-1}$ , membrane resistance is inversely related to the number of ion channels open. In other words, resistance goes up when channels close and goes down when channels open. Now simulate opening more ion channels in the membrane by adding a resistor to the circuit (thus decreasing the resistance between pin 13 and pin A5). How does this affect the amount of time it takes for the "membrane" to voltage to change (dV = 5 volts)? (See Figure 3B).
- Subthreshold depolarizing events combine to bring neuronal membranes to the threshold to fire an action potential. Both IPSPs and EPSPs will sum algebraically. When the summation occurs because the frequency of events is too rapid for one event to fully dissipate before the next occurs, this is called temporal summation. When the summation occurs because simultaneous subthreshold events are happening in different passive compartments of the cell but are working together to bring the spike initiation zone closer toward impulse threshold, this is spatial summation. Because there is only one voltage source in the 3D printed circuit, it can be used to illustrate temporal but not spatial summation. Use the 100 nF capacitor and one 100 M $\Omega$  resistor. In the Arduino program, change the pulse duration to 150 ms and the duty cycle (the fraction of the cycle period during which the voltage is on) to 0.75. Change the number of pulses per train to 5. Send the revised program to the Arduino using the right arrow button in the top left of the Arduino window. Observe the temporal summation. It should look similar to Figure 5. What do you think will happen if you remove the capacitor? What about if you change the time constant (by changing capacitors or adding/removing resistors)?
- Explain how temporal summation depends both on the membrane time constant and on the frequency of stimulation. At what frequency of stimulation do you begin to see temporal summation?
- A low-pass filter is a system that passes low frequency stimulation but blocks high frequencies from getting through. A high-pass filter is a system that permits high

frequencies but blocks low frequencies. Does the property of temporal summation make the neuronal membrane act like a low-pass or a high-pass filter? Explain.

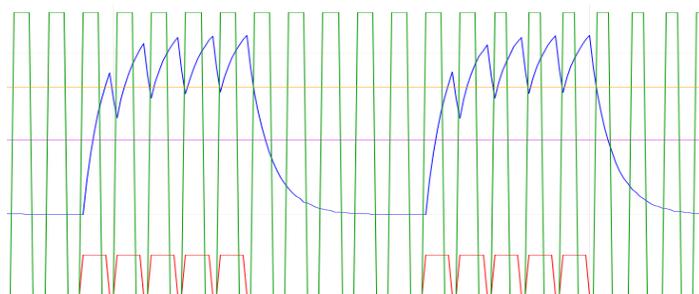
## DISCUSSION

Despite the great potential in improving learning, the EC is still an underused resource in undergraduate neurosciences courses. Different approaches to employ this model in the teaching of undergraduates has included solderless circuits and software implementations (as an example see the excellent model and laboratory exercises available from Amrita University at <https://vlab.amrita.edu/index.php?sub=3&brch=43&sim=129&cnt=1>). The ease and convenience of a 3D printed RC circuit like this rests in the ability of students simply to plug-in components without worrying about specific holes on solderless breadboards. In addition, it is neater and less subject to wire movement noise than circuits assembled with alligator clips. Finally, it permits students to visualize the circuit in a parallel configuration without keeping track of which wire is which.

We have also demonstrated the use of the Arduino as a very cheap combined programmable stimulator and oscilloscope. The Serial Plotter feature has several shortcomings, such as the fixed 500 data point screen width, the lack of an x-axis in time and no freeze-screen option. However, we have presented three effective solutions for these challenges that transform this relatively simple plotting tool into a digital chart recorder.



**Figure 4.** Effect of membrane capacitance on the charging time of the membrane. *Upper trace:* In this configuration, there is a 100 M $\Omega$  resistor at position R1 but both positions R2 and C are open. With the capacitor removed, it is clear that the voltage measured at A5 changes instantaneously with the voltage change at pin 13. *Lower trace:* The configuration has a 100 M $\Omega$  resistor at position R1, position R2 is open, and there is a 1 $\mu$ F capacitor at the C position. Compare these traces to the upper panel of Figure 2, which also has a total resistance of 100 M $\Omega$ .



**Figure 5.** A demonstration of temporal summation using the project. The settings include a pulse duration of 50 ms, a duty cycle of 0.75 and 5 pulses per train.

Our EC fulfills several critical learning objectives (for an extensive discussion see Dabrowski et al., 2013), but perhaps the strongest feature of this approach is simply that it teaches students basic neurophysiology while introducing them to key DIY innovation tools. Exposure to a wide range of tools for creative problem-solving is both a key principal of a liberal arts education and an essential component of professional development within the sciences. While the assessment of interdisciplinary learning can be challenging, some evidence points to a perception by undergraduates that neuroscience is a partnership between fields as opposed to an integrated, multi-field effort. In one study, students were asked to score neuroscience-related terms (like pH, behavior and reaction time) as to their relevance to various fields (chemistry, biology, psychology, physics). Analysis of the scores revealed clusters of terms organized in traditional knowledge domains, such as biochemistry, physics and psychology (Crisp & Muir, 2012). Most significantly for the present study, terms seen by the students as related to math and physics tended to be only distally related to terms related to biology. Exercises like the one described here have the potential to teach the students about the vital interrelatedness and interdependencies of scientific fields.

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## APPENDIX: Arduino Script

```

/* ----- */
//USER VARIABLES:
const int pulse_duration = 1000; // ms
const float duty_cycle = 0.5;
const int pulses_per_train = 1;
/* ----- */

#define CYCLE_PER int(pulse_duration/duty_cycle)
#define TRAIN_DUR CYCLE_PER*pulses_per_train
#define TRIGGER pulses_per_train==1?((millis()% \
CYCLE_PER)<pulse_duration):((millis()%CYCLE_PER) \
<pulse_duration)&&((millis()% (2*TRAIN_DUR))<TRAIN_DUR)

void setup() {
  Serial.begin(9600);
  pinMode(LED_BUILTIN, OUTPUT);
  pinMode(2, INPUT);
}

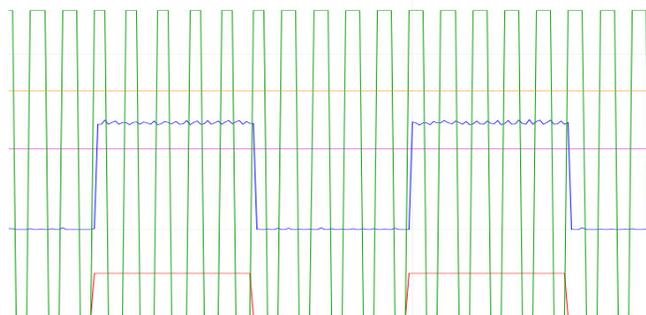
void loop() {
  float voltage = (analogRead(A5)/1024.0)*5.0;
  Serial.print(voltage);
  Serial.print(" ");
  if (TRIGGER) {
    digitalWrite(LED_BUILTIN, HIGH);
    Serial.print(-1);
  } else {
    digitalWrite(LED_BUILTIN, LOW);
    Serial.print(-2);
  }
  Serial.print(" ");
  (millis()%200)<100?Serial.print(5):Serial.print(-2);
  Serial.println();
  while (digitalRead(2)==HIGH)
  {
    // do nothing
  }
}

```

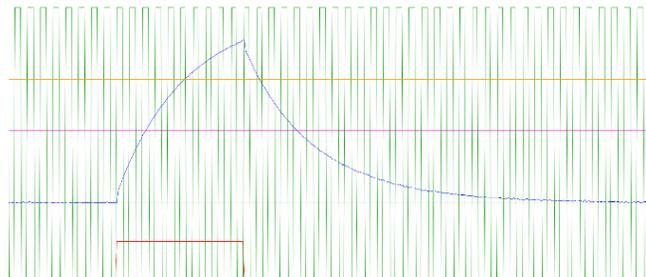
### Guide to the Student Exercises

- In this exercise, the student is asked to measure and calculate the time constant. The vertical green lines must be used as a reference for the time scale, and they represent divisions of 100 ms. Together with the horizontal orange and purple lines, estimating the time constant should present little problem. With respect to the calculations, encourage the students to do their calculations in base units (Farads and Ohms) so that their calculated figure comes out in seconds. By multiplying the values of the resistor and the capacitor, they will arrive at a time constant of 100 ms. However, what they measure will be slightly longer (roughly 120 ms), owing to series resistance in the circuit. The conductive paint (unlike wire) has resistance, and adds by our measurements ~120 kOhms of resistance to the circuit in the single-resistor configuration, with all resistances in series. These values may be different depending on the make and age of the conductive paint, so it is best to measure the total resistance in the circuit in both configurations between the wire that will connect to A5 and the voltage source (pin 13). It seems suddenly like a very complicated circuit, but all of the series resistances can be summed to a single resistance (Thévenin's Theorem) which can then be multiplied by the capacitor value to calculate the time constant. Be sure to remind the students that while resistances in series sum, resistances in parallel must be dealt with differently:  $R_{total}^{-1} = R_1^{-1} + R_2^{-1}$ . We measured time constants of 125 ms for the single resistor. Estimating 150 kOhms of stray series resistance, we calculated that the time constants should be 120 ms for the single resistor.

- It stands to reason that with the capacitor removed, the voltage flickers back and forth between 0 and 5 volts. This is because in this configuration there is simply resistance between pins 13 and A5 of the Arduino. The changes are instantaneous, but the sampling rate of the Arduino makes them look almost instantaneous. Some students with a little more exposure to electronics may ask why if there are multiple resistances in series the circuit isn't acting like a voltage divider in this configuration. A voltage divider can quickly be demonstrated by placing the second resistor in the capacitor place on the printed board. Because the voltage drop is being measured across one equal value, two resistors in series (instead of across the entire series resistance), the voltage shown on the Serial Plotter will now be half:



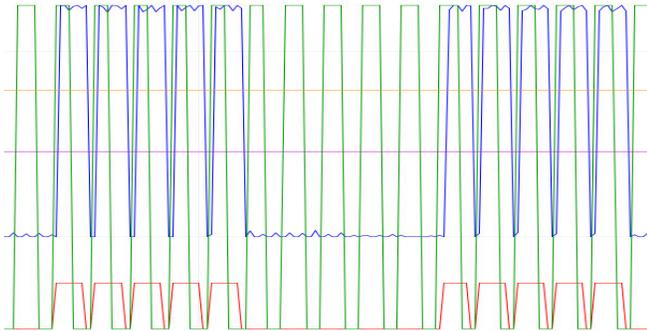
- By increasing the capacitance, they should be lengthening the time constant. The time constant we measured in this configuration was 1100 ms. However, it is necessary to alter the Arduino code to measure this correctly, because careful inspection of Figure 4B will reveal that the capacitor is never fully discharged with these stimulus parameters, and hence the time constant will be grossly underestimated. The physics here relate to temporal summation, which will be addressed explicitly in later exercises. By changing the pulse duration to 2000 ms and reducing the cycle period to 0.2, the time constant can be measured from the charging curve. (The pulse duration would have to be longer still to measure it from the discharging curve, and this could be problematic because the Serial Plotter only plots approximately 500 data samples). As soon as the code is changed, it can be sent to the board with the right arrow button in the Arduino GUI. Note, however, that if the jumper is in place between pins 1 and 3.3V or 5V on the Arduino (the "switch" for freezing the Serial Plotter so measurements can be made), the new program may not be sent correctly to the Arduino. It is best to disconnect that jumper whenever code is sent to the board. It is recommended to restore the original stimulation parameters before proceeding to the next exercise.



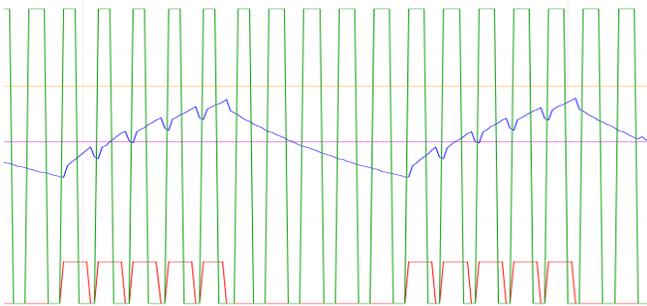
- We measured ~140 kOhms of stray series resistance in the circuit in the two-resistor configuration (see the solution to Exercise 1), but in the parallel resistor configuration, there is approximately 20 kOhms of resistance in parallel. Thus, we measured a time constant of 75 ms for the two resistor configuration and calculated 70 for this arrangement once

stray series and parallel resistances were accounted for.

5. The key to this exercise is to understand that the capacitor fails to discharge completely between pulses. If, for example, they have the configuration depicted in Figure 5, and they remove the capacitor, they will see the following:

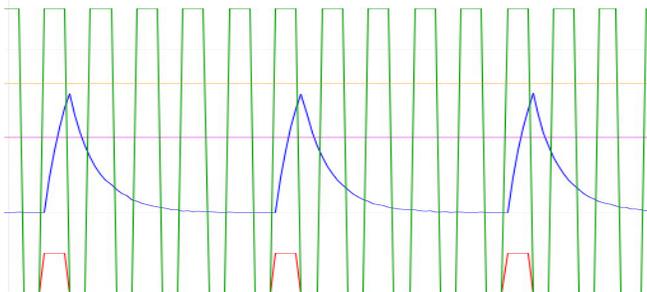


If, on the other hand, they substitute the larger capacitor (the #105), they will see this:

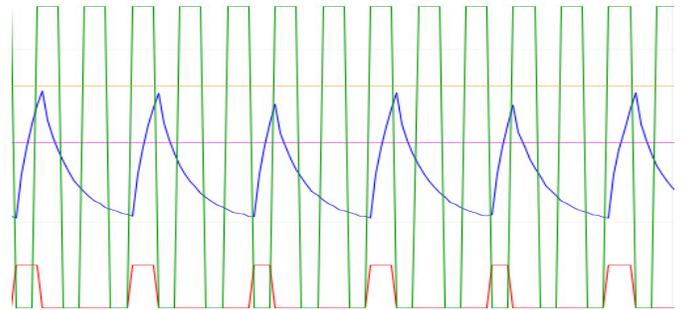


From these observations, they should be able to deduce that the resistor influences how fast the capacitor charges and discharges, but the summation occurs because the capacitor is already partly charged (i.e., there is already an electrical potential difference between Arduino pins 13 and A5 owing to the voltage stored across the capacitor) when the next stimulus comes, and so each successive stimulus builds on the preceding one.

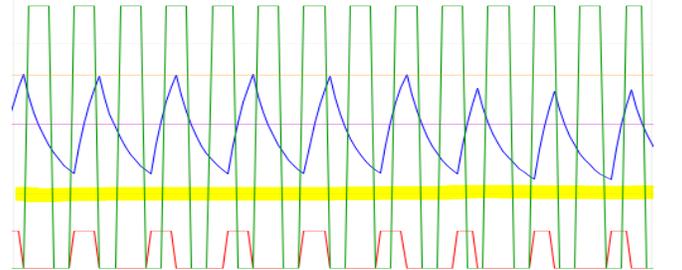
6. Imagine that the cell is depolarized to some voltage  $V_1$ . As soon as the stimulus stops,  $V_1$  begins to dissipate exponentially back down to rest. If the next stimulus doesn't come for a delay of about five time constants, there will be no summation. However, if another stimulus comes within that window, some summation will occur. In other words, you should see temporal summation for frequencies higher than  $[1 / (5 * \tau)]$  Hz. Frequencies lower than this will not lead to summation. This principle can be demonstrated by setting the pulse duration to 100 ms and the duty cycle to 0.1:



The frequency of stimuli here can be measured as 1 Hz. The frequency can be doubled by setting the duty cycle to 0.2



and we are now near the threshold for summation. Since the time constant is about 120 ms, the threshold is about 600 ms between stimuli (or 1.7 Hz). Doubling the stimulation frequency a second time (duty cycle = 0.4) delivers dramatic temporal summation



as evidenced by the incomplete discharge of the capacitor. Note that the curve never reaches the gray zero line (highlighted in the above panel), and also note the steepness of the discharge curve when the next stimulus arrives, compared to the nearly asymptotic curve in the 2 Hz example above. Note that the code is written to hold the pulse duration constant, so manipulating the duty cycle is simply adjusting the inter-pulse interval in proportion to the duration of the pulse.

7. Since lower frequencies of stimulation fail to result in temporal summation, but higher frequencies do generate temporal summation, the nerve membrane is behaving as a high-pass filter. The "pass" part of the filter of course means that an action potential is generated and the message is passed down the axon and across synapses. Temporal summation that fails to reach action potential threshold is being integrated but not "passed" through the filter.