

## Action Potential Exercise

This exercise will allow you to explore the properties of an axon in a simulation and thereby enhance your understanding of how the action potential works. Go to the Nerve simulation at <http://nerve.bsd.uchicago.edu/nerve1.html>. This is a simulation that models the properties of the squid giant axon of Hodgkin and Huxley fame.

**Passive axons.** First we will look at the properties of passive axons, that is, axons whose ion permeability does not change with time or membrane potential. Starting with the default parameters, click the “Axon Parameters” button. In the panel that appears, set “Max gNa” and “Max gK” to zero. Now click on “V vs t –Propagated AP”. The window that comes up shows the membrane potential  $V_m$  as a function of time in response to a square wave voltage stimulus. The stimulus is administered by the solid red electrode at distance = 0 cm as illustrated at the top of the window. The stimulus itself is the upper red trace on the graph. Three electrodes measure the membrane potential in response to the stimulus: the black electrode at 0 cm, the red at 5 cm and the blue wherever you set it by clicking and dragging it along the axon. The respective traces corresponding to the three recording electrodes are shown in the chart in black, blue and red (lower trace).

Click the start button and see what happens. Cable Theory says the axon should exhibit some voltage attenuation. Does it? By moving the blue recording electrode, try to find where on the axon you can start to see a change in membrane potential in response to the stimulus. How far from the stimulus is the recording electrode? Now use your understanding of Cable Theory and the blue recording electrode to estimate the lambda parameter for this axon. Record the value you got in the Action Potential quiz (Question 1). Click the “Axon –F6” button in the upper right. In the window that appears you will find the radius ( $a$ ) and the internal resistance ( $R_i$ ) of the axon. Given these values and your estimate of lambda, estimate the membrane resistance  $R_m$  and record it in the quiz (Question 1). One of the parameters in the Axon Parameters window is the leak conductance, “Max gL” (lower right). gL is the conductance of the membrane. It should be (roughly) the reciprocal of the resistance  $R_m$  that you calculated. Is it? (Question 1)

Play around with the axon parameters and see if they have the effects that you expect.

**Action Potential – the time dimension.** Now we will see what happens in an active neuron, one where the sodium and potassium conductances change with time and  $V_m$ . Restore all of the parameters to their default values. Open the “V vs t –Propagated AP” window and press “Start-F3” in the upper right. You will see the change in membrane potential as a function of time at each point along the axon where there is a recording electrode. Does Cable Theory still accurately predict the behavior of the axon (quiz Question 2)? What Cable Theory predictions fail (Question 2)?

**Action potential propagation.** What you should see is a traveling wave of depolarization referred to as the action potential. Which direction is the action potential wave moving (Question 3)? How fast is it moving? Is it accelerating, decelerating or moving at a constant speed (Question 3)?

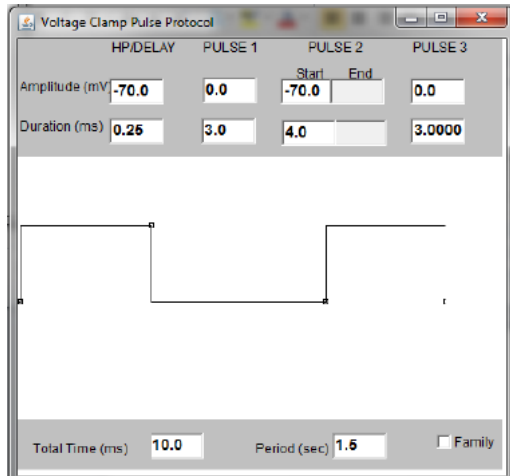
**Threshold.** Click “PULSES-F7” in the upper right and in the window that comes up, try different combinations of “Pulse 1” amplitude and duration (you can click and drag to square wave in the window to do this). You may want to increase the total time at the bottom. If you enter a number by keyboard, don’t forget to press “enter”. What do you suppose determines whether the initial stimulus induces an action potential? Is there a maximum stimulus amplitude that will never result in an action potential, no matter how long the duration? What determines this maximum (Question 4)?

**Ionic components.** Go to the Axon-F6 window and set “Max gNa” to zero. What happens when you apply a stimulus (Question 5)? Under the default conditions, what do you think is happening to gNa when you apply the stimulus? How could you test this? Starting from the default, set “Max gK” to zero. What happens even before you apply the stimulus (Question 5)? Why?

**Voltage Clamp.** To really understand the roles of each of the ion conductances in the action potential, you need to be able to isolate the effect of the membrane potential on the conductance from the effect of the conductance on the membrane potential. Otherwise there is no way to separate cause and effect. To do this we can use electronics to clamp the membrane at a desired potential (a technique called “voltage clamp”) and look at the effect of membrane potential on conductance. Close your previous window and return to your default values. Now open the “Voltage Clamp” window. This is a static plot of the membrane current (red) in response to a square wave membrane depolarization (black). Time is on the x-axis. First determine the respective contributions of sodium and potassium on current but setting, in turn, the gNa and gK to zero (in “axon parameters”).

With gK set to zero, notice that the depolarization provokes a sodium current that rises and falls, even though the membrane potential remains depolarized. So the depolarization of the membrane provokes a *voltage-activated* gNa – one that spontaneously *inactivates* over time if the voltage remains high. Click “pulses-F7” to bring up the “Voltage Clamp Pulse Protocol” window. Click and drag the square wave to simulate greater or lesser depolarizations with your voltage clamp and pay attention to how the peak Na current changes. Can you explain why there is no current at small depolarizations, then increasing inward current (positive charge moving into the cell) then less inward current then outward current (Question 6)? Why is current zero at two different depolarizations? In the “CONC-F8” window change the [Na]<sub>i</sub> and look what happens when you change V<sub>m</sub>. What effect does [Na]<sub>i</sub> have? Does this give you any clues to why the current goes to zero (Question 6)?

Return to your default values and now do the same series of membrane potential changes except pay attention to the *rate* at which the sodium current inactivates. What does rate of inactivation depend on (Question 7)? The maximum sodium current? The maximum depolarization? The direction of current? To better understand what’s going on, create a protocol that has two depolarizations: the first to zero V<sub>m</sub> and lasting three milliseconds, and a second square pulse, same values, except four milliseconds after the first.



Does the current response look the same for both pulses? Play around with the size and duration of the first square wave and look at the effects on the second square wave. What determines the amplitude of the second depolarization?

Return to your default values. Open the “CONC-F8” window. There you can adjust the concentrations for “TTX” and “Pronase”. In the voltage clamp window try a series of virtual experiments to determine what TTX and Pronase do (Question 8).

**Action Potential – the distance dimension.** Close your active window, return to the default values, and open the “V vs x – Propagated AP” window. This simulates what the membrane potential does at all points along the axon over time during an action potential. (We don’t actually have a way of measuring the membrane potential at all points simultaneously, but this shows what we predict it would look like.) Does the axon behave the way you expected? Does it surprise you that action potentials are traveling both directions (Question 9)?