**Squid Axon Action Potential Simulations[[1]](#footnote-1)**

**Introduction:**

 This simulation uses two groups of equations: one set models the response of the membrane to changes in voltage (it is essentially a model of membrane resistance and capacitance). The other group involves (i) a series of equations developed by Hodgkin and Huxley that predict the kinetics of the Na+ and K+ gates; (ii) equations that calculate K+ and Na+ conductances (*G*K+ and *G*Na+ ); and finally the Goldman-Field Equation is used to predict membrane potential. This potential in turn is used to solve the equations for the next iteration of conditions (conditions change with time since the voltage history has effects on the gates -- remember the ball and chain model for instance of the Na+ gates). Iterations are made for constant time intervals (0.01 msec/interval). Thus, equations that accurately describe the orderly operation of the Na+ and K+ gates in a constant ionic environment predict the events of an action potential.

This model consists of:

• **Controls that permit the experimenter to adjust the characteristics of two stimuli.** These characteristics are:

**1. Strength** (self-explanatory)

**2. Duration** (self-explanatory)

**3. Delay**. This is the time between the onset of the first and second stimulus. Since the first stimulus is taken as time zero (the simulation triggers on this stimulus) the delay is also the elapsed time between the start of the sweep and administration of the second stimulus. The delay can be any length of time but if it is over 10 msec, the second stimulus will not appear in the experiment; nor will it roll over and appear on the next screen. Thus, selecting a delay >= to 10 msec is a technique to use a single stimulus in an experiment.

* Displays of *E*M and *G*K+ and *G*Na+ vs. time. There is a picture of the stimulator control panel for the stimulator on the top of the next page. The pictures below are of the *E*M and stimulus plots and then the *G* plots and the controls.



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**Uses of the Model**

 Like any model, this model is NOT REALITY. Yet in many aspects it mimics reality effectively. Compare what you observe with what you have learned about active and passive responses of neurons. Take good notes of what you see and note especially how well the model works. You will perform a series of different groups of experiments.

**Experiments**:

 (1) The first will involve **finding the threshold**. In the search for the threshold, you should also observe electrotonic and local responses. **Use a duration of 2 msec for your determination of threshold.** Decide on an orderly process that will allow you to find the threshold. List the elements that such a search should have (i.e., how should the search be conducted?)

 (2) Determine the **length of the absolute and relative refractory periods** (ARP and RRP). You will need to find the ARP period first. What controls will you manipulate?

 (3) **Strength-Duration curve**. Starting with your original threshold at 2 msec, systematically change strength and duration and determine the strength duration curve. This is defined as the voltage threshold for a given duration. Find at least 4 points above and four points below your 2 msec duration. Label the chronaxie and rheobase – what are the utility of these two measurements.

 (4) **Effects of stimulus strength on firing rate**: Using a single stimulus of 10 msec duration, determine (quantitatively) the relationship between firing rate (APs per 10 msec) and strength. I suggest looking at the following stimulus levels: 0.5, 1,2,4,6,8,10,12,16,20,25,30,35,40, & 50 mV. Count anything that looks remotely like an AP as an AP – at the higher stimulus levels the simulation will start to be a bit weird but it will still make a valid point. Try to explain this relationship both in terms of the mechanism that causes it and information that is encoded. What parameter that you measured earlier should set the maximum firing rate? Did it?

 (5) **Critique the model**. Where did it succeed and fail?

**Using the Model**

1. Boot the Macintosh.

2. Find the Icons called **Squid AP simulator** and **Squid AP analysis. Click on one, hold the shift key down and double click on the other.** This will allow you to start both of the VIs you will need at the same time.

3. Once the program is up and running use the **Window** menu selection to move between the two VIs.

4. Click on the **Tools** menu and click down on the top of the toolbox that appears. Drag the box off the menu. It will look like this:



The tools you will need to use are the **pointing finger** (for changing dial settings), the **hand** (for moving things around -- please only move the toolbox; don't resize or move the graphs or stimulator), and the **clipboard** (for moving refractory period data to the analysis VI).

4. Stimulator parameters can be set using the pointing finger tool either by turning the dial or by click dragging the tool across the digital display below each dial. The display will become highlighted and the exact value can then be entered by typing followed with a return.

5. Under the **OPERATE** menu item, there is a choice called "Set all to default values". Use this if you wish to return to original stimulator values.  **Please do not re-set any default values**.

6. To run the program after you have entered all of the stimulator settings you wish to use either:

 (i) Press command r (The key with the apple (next to the space bar) and r held down at the same time. -- **or**

(ii)press the **arrow icon** as shown below:

 

(not the arrow chasing the arrow -- if you do that, press the stop sign button. This is shown below:



 -- **or**

 (iii) Select **Run** from the **OPERATE** menu item.

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Electrophysiology **Lab Report #1**

Fall 2016

**35 points**

This is a team project – one paper per team both people get the same grade. You should feel free to discuss this material with other members of the class; equally, you are free to replicate any experiment on the simulation. However, when you write the answers to these questions, this is to be your own team's work. It will be easiest if you use excel for graphing and simply answer the questions below.

1. For an initial stimulus of 2 msec duration, what was the threshold? Did you see any sign of a local response below threshold? Briefly explain.

2. In a couple of sentences explain how you determined absolute and relative refractory periods? List the lengths of each?

3. Plot your strength-duration curve. Find out (from a library source) what the terms **chronaxie** and **rheobase** mean (and perhaps their medical uses) and show them on your plot.

4. Using up to date primary or secondary sources, explain the shape of the strength-duration curve. Be sure to cite your references.

5. What effect does increase in stimulus strength have on the time between the initiation of the stimulus and an active response? Explain.

6. (a) Make a plot of the relationship between stimulus intensity and firing rate (for a continuous stimulus) using the suggested stimulus values. Also make a ln vs. ln plot of the same

(b) In a couple of paragraphs relate this to the translation of information from the physical world (stimulus) to a neural representation -- how faithful is this translation? Discuss the analog nature of the stimulus and the nature of the neural representation as part of your answer.

7. What were the shortcomings of the model? Where did you feel that it was particularly unreal in its portrayal of neural activity? ( paragraph and list)

8. Predict the effect of lowering the temperature on the general shape of the active potential. The representations you see are for 25o C, far above the temperatures that Hodgkin and Huxley used to determine the kinetics of the Na+ and K+ channels (they used about 4oC). Make a sketch.

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