Neurophysiology
IV. Electrophysiology of the Neuron
Software Simulation

Materials

NeuralSim Software - A Computerized Interactive Study Tool [1]

Purpose

To investigate the quantitative behavior of action potentials and post-synaptic potentials and their qualitative changes in response to different membrane properties and ion concentrations.

Introduction

The dynamic nature of excitable cells makes it difficult to fully appreciate their behavior using standard textbook approaches. In this lab we will investigate action potentials and synaptic potentials using a computer simulation. The simulator accurately reproduces the behavior of excitable cells based on the mathematical model of Hodgkin and Huxley [2] and presents the results graphically. This will allow you to concentrate on the concepts without getting tied up in the mathematical details.

We will be using the APSIM software, which allows you to simulate the stimulation of an excitable cell. We will introduce its basic function and describe the menu options you will need for today’s lab, after which you should work through the guided exercises provided. These exercises should be completed and turned in at the end of the lab. When you are finished with the required exercises, you may go back and explore other aspects of the simulator if you wish to do so (a list of possible topics is listed at the end of the handout). A second program is included with NeuralSim, PSPSIM, which you are encouraged to try if you have time. This program simulates the behavior of a post-synaptic cell in response to excitatory and inhibitory synaptic potentials. The operation of the two programs is very similar, and you should have little trouble using PSPSIM once you understand APSIM.

APSIM - Action Potential Simulator

The APSIM software allows you to simulate the stimulation of an excitable cell and investigate quantitatively the behavior of the action potential. Behind the scenes are the actual equations derived by Hodgkin-Huxley in their now-classic experiments on the giant squid axon.

The APSIM main window displays the results of running a given simulation. This consists of a stimulating current pulse at bottom (in blue) and plot of the membrane potential. The

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1 Contributed by M. Jones, Spring 1996
stimulus strength and duration can be adjusted using the slider bars. To the left of the main display are the three buttons that control simulation execution: Erase, Run, and Halt. Run begins a simulation run. Halt stops a running simulation. Erase clears the screen.

APSIM also has a number of pull down menus for changing membrane properties, making measurements, plotting additional parameters and so forth. Many of these features will not be used in todays lab; those important for the lab exercises are shown below in **boldface**:

**File**
- Close
- Run
- Halt
- **Quit** - Quit the Program

**Edit**
- **Mode**
  - **Membrane** - Set active or passive membrane
  - **No. Pulses** - Specify one or two stimulating pulses

**Params**
- Maximal Conductances
- Ion Concentrations
- Gating Kinetics
- **Timebase** - Expand or contract the time scale of the main window
- Plot Scaling
- **Reset All Params** - Set all parameters to their startup values

**Plots**
- Membrane Currents
- Membrane Conductances
- Channel Gates
- **Measure** - Bring up the measurement window
- Experiments
APSIM Exercises*
Name: _____________________________
Student ID: ________________________

Notes:

1) In these exercises, *current* values are measured in µAmps, and are listed at the right of the slide bar, whereas *potential* is measured in mV, and is measured using the cross-hairs brought up by the Measure menu. Any numerical answers you provide must include the correct units!

2) Note that the membrane resting potential is given at the left of the display (under the control buttons) labeled “R.P.”. The 55 mV and -80 mV listed at the left of the main window in bold represent the Nernst potentials of sodium and potassium, respectively.

3) You may need a calculator for some of the questions. If you don’t have one with you, select the “calculator” application from the apple menu. Ask your TA if you need help.

A. Threshold Potential

Determine the neuron’s threshold potential using a 0.5 msec stimulating current pulse. (Make sure you have selected Active Membrane from the Mode menu. Choose the 1 pulse option from the Mode menu and make sure the timebase is set to 2 msec (use the Time Base item in the Params menu if necessary). Use the Stimulus Strength scroll bar to adjust the strength (i.e. amplitude) of the current pulse until the pulse just elicits an action potential.

A.1) What is the amplitude of the smallest current pulse that elicits an action potential? ______________________

To determine the membrane threshold potential, decrease the strength of the stimulus by one “click” so that stimulation fails to elicit an action potential, open the Measure window from the menu bar and use the cross hair to read the voltage at the end of the 0.5 msec stimulus.

A.2) What is the value of the membrane threshold potential? __________________

Increase once again the current pulse so that an action potential is generated. Open the Membrane Conductances and Membrane Currents windows from the Plots menu. This will display the changes in sodium and potassium conductances and currents over the course of the simulation, and show you how changes in conductance control the rising and falling phases of the action potential. The Membrane Conductances displays sodium permeability in red, and potassium permeability in blue. Click “View Vm” to have APSim include membrane potential in this plot. The Membrane Currents window shows sodium current in red and potassium current in blue. Down is current flow into the cell (i.e. depolarization). The green line is capacitive current and may be ignored, as can the small spike that occurs in the sodium current. Drag the windows around until they line up underneath one another. You may want to make sketches of these windows, which you can refer to outside of lab.
A.3) Why does sodium conductance rapidly decline after the action potential reaches its peak?

A.4) Why does sodium current continue to increase even as the sodium conductance declines?

A.5) Why does the K current decline more rapidly than the K conductance?
B. Parameters of the Action Potential

Measure the peak voltage of the action potential by centering the cross hairs on the peak.

B.1) What is the value of the action potential peak? ______________________

B.2) Why doesn’t this value equal $E_{Na}$?

Now measure the most negative potential reached during the hyperpolarization-overshoot that follows repolarization of the action potential.

B.3) What is the value of the membrane potential? ________________________________

B.4) Why doesn’t this value equal $E_K$?

B.5) Why is this value more negative than the resting potential?

The duration of the action potential is defined as the time it takes for the action potential to go from 50% of its full amplitude on the rising phase back to this potential during the falling phase.

B.6) Compute the amplitude of the action potential. This is equal to the peak value (question B.1) minus the resting membrane potential (R.P.) _____________________

B.7) What value of the membrane potential represents 50% of the action potential amplitude?

B.8) Using the cross hairs, determine the latency ($t$) at which the membrane potential reaches the value obtained in B.7 during the rising phase: ___________________
B.9) Using the cross hairs, determine the latency (t) at which the membrane potential reaches the value obtained in B.7 during the falling phase: ___________________

B.10) What is the duration of the action potential? ___________________
C. Refractory Period

Set the 2 pulses option from the Mode -> No. Pulses menu. Set the Time Base to 4 msec using the Params -> Time Base... option under the Params menu.

Adjust Pulse #1 so that it is 30 µAmps in amplitude and 0.5 msec in duration. Then, click on the Pulse # button in the main window (at the lower left side of screen above the current pulse scroll bars) until the label on the current pulse scroll bar reads Pulse 2. Set the Pulse 2 amplitude to 100 µAmps and its duration to 1.0 msec. Click on the Pulse # button until the current scroll bar label reads Interpulse Interval. Start with an interpulse interval of 5 msec. Click Run.

C.1) How many action potentials are generated? _________

Keep increasing the interpulse interval in steps of 1 msec until you elicit an action potential with the second pulse, at which point refine your adjustment of the interpulse interval to 0.2 msec increments. For our purposes, define the refractory period to be equal to the interpulse interval.

C.2) What is the value of the refractory period? _______________________________

C.3) What would the maximum firing rate (number of action potentials per second) of this neuron be? __________________________

References


ANSWERS:

Question A.1)

18.20 µAmps

Question A.2)

-55 mV

Question A.3)

The rising phase of the action potential is generated by the opening of voltage sensitive Na⁺ channels in the membrane that allow Na⁺ to move down its concentration gradient (established by the Na⁺/K⁺ pump) and into the cell. However, when the action potential reaches its peak, the voltage sensitive Na⁺ channels close and, thus, Na⁺ conductance is drastically decreased.

Question A.4)

The answer to this question is based on the premise that current (ionic flow) is a function of conductance (channels) plus driving force (electro-chemical gradient).

The question states that Na⁺ conductance is declining while Na⁺ current is increasing. What this means is that the voltage sensitive Na⁺ channels are closing (decreasing conductance), yet more Na⁺ ions are flowing across the membrane (increasing current). In the above premise, the only variable left that could be responsible for increasing Na⁺ current is the electro-chemical gradient. Knowing this, we can proceed with the answer to the question.

Na⁺ is under the largest chemical pressure to enter the cell at the very beginning of the action potential because the Na⁺/K⁺ pump has been busy working to keep the majority of Na⁺ on the outside of the cell. The question is asking about the later stages of the action potential when Na⁺ conductance is declining, so we can eliminate chemical pressure as a reason for the increasing Na⁺ current.

That leaves the electrical gradient as the probable culprit (something must be drawing more Na⁺ into the cell). To explain this phenomenon, we must consider another ion species, namely K⁺. At the end of the rising phase of the action potential, the voltage sensitive K⁺ channels begin to open (the delayed rectifier). This lets K⁺ ions flow down their concentration gradient (also established by the Na⁺/K⁺ pump) and OUT of the cell. Because K⁺ is a positive ion, when it flows out of the cell, the inside of the cell becomes more negative. This increase in negativity draws more positive Na⁺ ions into the cell despite decreasing Na⁺ conductance. Here you have the answer to this question.

Question A.5)
This question is also based on the premise that current (ionic flow) is a function of conductance (channels) plus driving force (electro-chemical gradient).

Unlike question A.4, this question states that K⁺ conductance is declining very slowly, yet K⁺ current is declining at a much faster rate. Transforming this into physiology, we know that K⁺ channels are closing very slowly yet the number of K⁺ ions flowing across the membrane is decreasing at a much faster rate.

With this information, we again know that the question hinges on K⁺’s electro-chemical gradient. The longer that the voltage sensitive K⁺ channels are open, the more time K⁺ has to diffuse across the membrane and approach its equilibrium potential. By the time the voltage sensitive K⁺ channels begin to close (and we observe a very slowly decreasing K⁺ conductance), the majority of K⁺ ions have already diffused across the membrane so there is far LESS pressure on the remaining K⁺ ions to flow. Less pressure equals less movement, and less movement equals less K⁺ current!

Question B.1)

44.5 mV (+/- 1 mV)

Question B.2)

If we did assume that the peak voltage of the action potential was equal to E_{Na}, we would not be considering how other ions species affect action potential dynamics, namely K⁺.

The peak voltage of the action potential doesn’t equal E_{Na} because voltage sensitive K⁺ channels begin to open BEFORE the voltage sensitive Na⁺ channels begin to close. Thus, toward the top of the rising phase of the action potential, positive Na⁺ ions are rushing into the cell at the same time as positive K⁺ ions are rushing out of the cell. Where these two ionic flows balance out is where the peak of the action potential is found.

Question B.3)

-78.8 mV (+/- 1 mV)

Question B.4)

Again, we MUST consider the dynamics of all of the ion species involved in the action potential. The hyperpolarization-overshoot phase does not equal E_{K} because K⁺ is not the only ion in motion, and its effects are tempered somewhat by the movement of Na⁺ into the cell and Cl⁻ out of the cell.

Question B.5)

There are two potential reasons for this overshoot.

FIRST, the most negative potential reached during the hyperpolarization-overshoot phase is more negative than the resting potential because the resting potential is generated by
the Na⁺/K⁺ pump and leakage currents, whereas the hyperpolarization-overshoot phase is created almost entirely by K⁺ moving down its concentration gradient and OUT of the cell through voltage sensitive K⁺ channels. The reason that hyperpolarization “overshoots” the resting potential is because the voltage sensitive K⁺ channels close too slowly to keep the membrane potential from stopping at the resting potential. This delayed closing allows the membrane potential to approximate Eₖ which is more negative than the resting potential.

SECOND, the Na⁺/K⁺ pump speeds up during the course of an action potential thereby causing the hyperpolarization-overshoot.

**Question B.6)**

\[44.5 \text{ mV} - (-62.7 \text{ mV}) = 107.2 \text{ mV}\]

**Question B.7)**

\[\frac{107.2 \text{ mV}}{2} = 53.75 \text{ mV}; \quad -62.7 \text{ mV} + 53.75 \text{ mV} = -8.95 \text{ mV}\]

**Question B.8)**

4.31 msec* to reach -9.1 mV

*Note: The resolution of the measurement device precludes an accurate measurement!

**Question B.9)**

5.78 msec to reach -9.1 mV.

**Question B.10)**

Duration of the action potential is defined as the time it takes for the action potential to go from 50% of its full amplitude on the rising phase back to this potential during the falling phase. Thus, to find the answer, you must take your answers from questions B.8 and B.9 and subtract them. The positive number (no such thing as negative time), is the duration of the action potential in msec.

\[5.78 \text{ msec} - 4.31 \text{ msec} = 1.47 \text{ msec}\]

**Question C.1)**

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**Question C.2)**
The refractory period is the time following an action potential during which another action potential CANNOT be generated. In this simulator, the refractory period is 5.40 msec (with an interpulse interval of 5.60 msec another action potential IS generated, so this is outside the refractory period).

**Question C.3)**

Assuming that another action potential can be generated every 5.60 msec (remember that the refractory period is 5.40 msec), and there is 1000 msec in a second, we know that, at most, 178 action potentials can be generated in one second.

"The actual number is 178.5714286, but we MUST round down to the nearest whole number because there is no such thing as 57% of an action potential in physiology (remember, it is an all-or-none phenomena)."