GENERATION OF ACTION POTENTIALS

Introduction

GENESIS (GEneral NEural SImulation System) is a general neuron simulation package developed at Caltech. Specifically, code has been developed using GENESIS to model the squid giant axon. The patch of excitable membrane is represented by the Hodgkin-Huxley model.
The model can be used to generate action potentials and has the following features:

- voltage and current clamp protocol
- current clamp protocol with two independent stimulating pulses which may be single shot or periodic
- manipulation of extracellular ion concentrations
- channel blockade
- ability to plot:
  - membrane current
  - membrane voltage
  - Na^+ and K^+ currents and conductances
  - state variables m, n, and h (Hodgkin-Huxley model)
  - state plots involving V_m, m, n, and h

You will carry out experiments as though you were making microelectrode recordings from a single squid axon, trying to recreate Hodgkin and Huxley's original experiments. Both voltage and current clamp modes will be investigated. The goal is to understand the ionic currents and conductances that underlie such typical neuronal behaviour as:

1. Firing of an action potential
2. Frequency encoding of stimulus amplitude
3. Accommodation
4. Refractoriness

Conventions:

Membrane Potential: Hodgkin and Huxley considered membrane potential as the displacement from the resting value. The modern convention is to measure the actual membrane potential. In keeping with modern convention, V_m (resting potential) is taken as -70 mV and depolarizations are positive going on the screen. See figure 1.

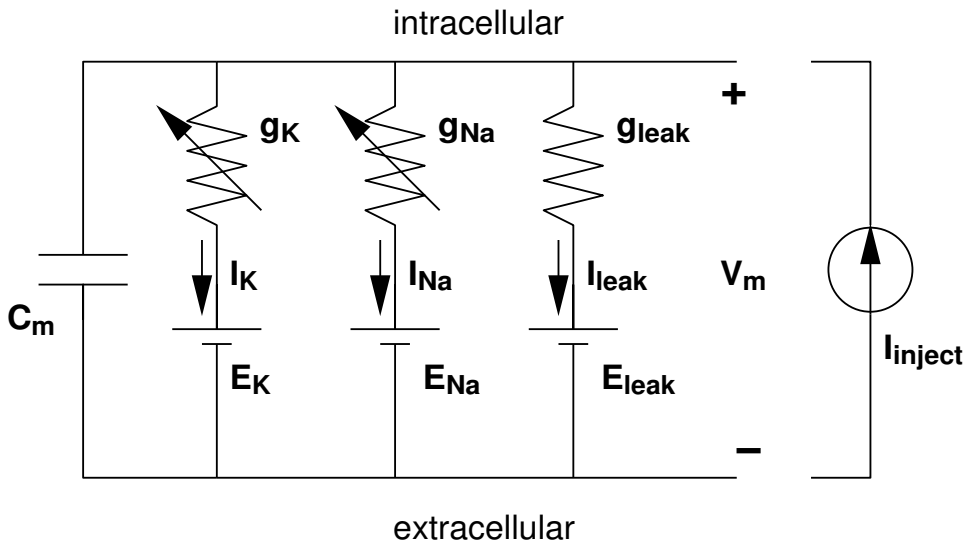

Figure 1: Equivalent circuit of membrane showing voltage and current conventions.

Currents:

1. Positive injected current pulses produce a net entry of cations which depolarize the membrane.

2. In Hodgkin Huxley conventions, (e.g. Na^+ entry) entry of cations into the cell is plotted as positive going-current. Modern convention inverts this and plots inward flow of cations as negative-going currents. We use modern convention; Na^+ entry is a negative-going current on the screen plot.

GENESIS

Running the program

To run the squid simulation on a sparc station, type the following commands:
host> **setenv LD_LIBRARY_PATH /local/X/lib**
host> **cd /local/packages/genesis2.0**
host> **cp .simrc ~**
host> **genesis**
genesis #0 >**cd Scripts/squid**
genesis #1 >**Squid**

Some messages will be printed in the shell window and five windows will pop up. The windows which pop up are called *forms*. In a form are several types of input/output areas called *widgets* which are explained below.

title This widget performs no action and merely displays the title of the form on a green background.

dialog The description is given on the left side while the right side contains the input field. The mouse must be moved to the right side and then text can be entered. **For a change to take effect, hit return after entering the new value *or* click on the left portion of the widget.**

button Clicking on the widget will cause the appropriate action to be performed.

toggle Clicking on the widget alternates between two states. The current state is displayed in the widget with one state being displayed on a blue background and the other on a red background.

graph XY graph of variables

Form Descriptions

popups

Widget	Type	Description
Help	button	pop up a window with scrollable help text
Phase Plot Hidden/Visible	toggle	hide/expose graphs of phase plot and HH parameters
Na channel blocked/unblocked	toggle	set conductance of Na channels to zero/nonzero
K channel blocked/unblocked	toggle	set conductance of K channels to zero/nonzero

Simulation Control

Widget	Type	Description
RESET	button	reset variables to initial conditions
RUN	button	start simulation with current conditions
STOP	button	stop simulation and preserve conditions
QUIT	button	pop up a confirmation window about exiting program
Time (msec)	dialog	length of simulation
dt (msec)	dialog	simulation time step
Toggle Vclamp/Iclamp Mode	toggle	set to voltage/current clamp paradigm
Overlay On/Off	toggle	Don't/Do clear graphs on RESET
Clear Graphs	button	clear curves from graphs

Current Clamp Mode

Widget	Type	Description
Base Current Level (uA)	dialog	basal current level in μA
Pulse 1 Current (uA)	dialog	pulse 1 amplitude in μA
Onset Delay 1 (ms)	dialog	time at which pulse 1 starts
Pulse 1 Width (ms)	dialog	pulse 1 duration in milliseconds
Pulse 2 Current (uA)	dialog	pulse 2 amplitude in μA
Onset Delay 2 (ms)	dialog	time from onset delay 1 at which pulse 2 starts; it must be greater than Pulse 1 Width
Pulse 2 Width (ms)	dialog	pulse 2 duration in milliseconds
Single Pulse/Pulse Train	toggle	pulses applied once/repeatedly

See figure 2 for graphical explanation.

External Concentrations

Widget	Type	Description
[K]	dialog	external K^+ concentration in millimolar
[Na]	dialog	external Na^+ concentration in millimolar
Default Values	button	reset concentrations to default values

Voltage Clamp Mode

Widget	Type	Description
Holding Voltage (mV)	dialog	basal V_m in millivolts
Holding Time (ms)	dialog	time to keep V_m at holding voltage
Prepulse Voltage (mV)	dialog	voltage applied before clamp
Prepulse Time (ms)	dialog	duration of prepulse
Clamp Voltage (mV)	dialog	clamping level
Clamp Time (ms)	dialog	duration of clamp

See figure 3 for graphical explanation.

Graph Scale

Widget	Type	Description
Done	button	hide form
xmin	dialog	minimum x value of graph in ms
xmax	dialog	maximum x value of graph in ms
ymin	dialog	minimum y value of graph
ymax	dialog	maximum y value of graph
overlay	dialog	0 = off, 1 = on

Important Notes:

- When a simulation is run, it must start with initialized values. This is accomplishing by the RESET button. *This must be done before every run.* Hitting RUN will cause the program to continue running from the current state for the time set by the *Time* widget.

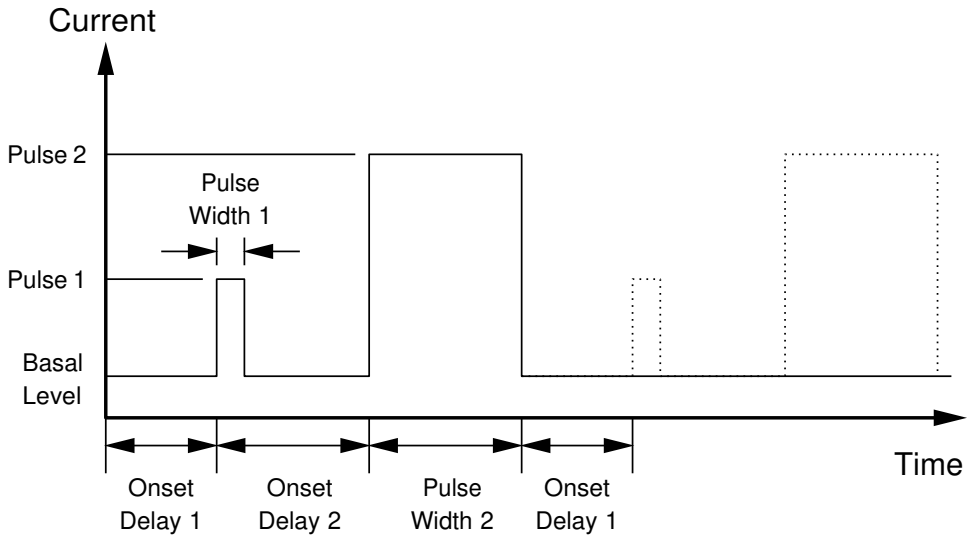

Figure 2: Definitions of quantities defining current clamp stimulus. If a pulse train is specified, the pulses continue *ad infinitum* as indicated by the dashed lines.

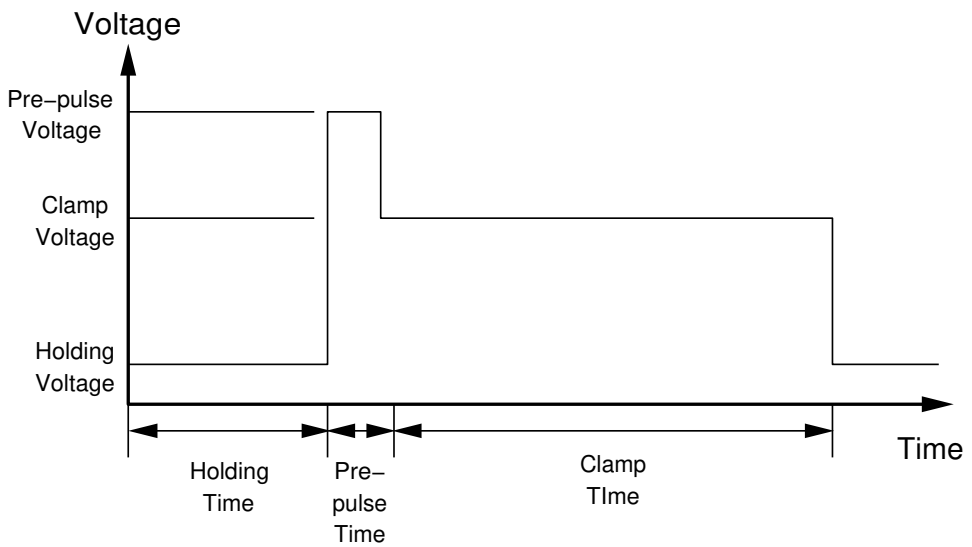

Figure 3: Definitions of quantities defining voltage clamp stimulus.

- The curves may be moved around the viewport by using the arrow keys. An area of the graph may be expanded by pressing a mouse button at the top left of the area to be zoomed and releasing it over the bottom right corner. Pressing "a" will scale the graph to show all data. Type *help xgraph* to see all options.
- To view the curves from several runs on the same graph, set *overlay* on.
- To hide the Phase plot widget, **do *not* use the window manager quit.** This will quit the entire program. Use the button on the *Simulation Control* form.
- **To print a graph**, another terminal window must be opened and a UNIX utility used, eg. type *xwd | xpr | lpr -Php1106* and click in the window you want printed. Remember that several curves may be plotted on one graph to reduce the number of graphs and make comparisons easier.
- To change the x-axis scale on all graphs, set a new *Time*. All axes will be scaled to this value.

EXPERIMENTS

The experiments will be run in two parts over two lab sessions. **A lab report is due for each part.**

Part 1

A. Action Potential

Set the *Pulse 1 Width* to 1 ms and observe an action potential by depressing the RUN button.

1. Describe the action potential, referring to characteristic such as threshold and amplitude, etc.
2. What seems to be the value of threshold?
3. What happens when you reduce the current pulse to 0.06 μA ?

Reduce the current pulse to 0.04 μA.

4. Describe the voltage response of the membrane.

If the amplitude is insufficient to trigger an action potential at a particular pulse width, it may be possible to trigger one by increasing the pulse width. This property will be fully explored later in another section.

B. Latency

Produce point by point a latency curve for the membrane as follows: For various amplitudes of stimuli, determine the minimum pulse width required for an action potential and plot the results.

1. Is there a simple numerical relationship between time and stimulus strength? **N.B.** dt must be less than the pulse width.

2. The *rheobase* is the maximum amplitude which cannot trigger an action potential regardless of duration. From your graph, what is its value?

3. From your graph, at what frequency would a stimulus of .05 μA be encoded if latency were the only consideration. What is the actual frequency?

C. Refractoriness

Nonlinear oscillators exhibit regions in their cycle were they are less susceptible to stimuli than at other times. If no stimulus, regardless of amplitude, has an effect, the system is said to be in an *absolute refractory period*. Following this is a *relative refractory period* where stimuli have effects but the amplitude must be increased to have the same effect as a stimulus applied outside the refractory period. In the case of our neuron, we will initiate an action potential and then immediately try to produce a second one.

1. Apply a $1\mu A$ pulse of 1 ms duration to the squid neuron. By application of a second pulse, of 0.1 ms duration, at a set delay after the first pulse, try to elicit an action potential. In such a manner, construct a graph of the minimum pulse 2 amplitude required to produce a second action potential for various delays.

2. How long is the absolute refractory period?

3. How long is the relative refractory period?

D. Passive Properties

Until now, all pulses have been depolarizing. The effect of hyperpolarizing stimuli is to be studied in this section. Choose a $-0.1\mu A$ stimulating step current (i.e. duration 100 ms) and observe the plot.

1. The membrane appears to respond in a passive manner. Can this initial hyperpolarizing portion be characterized by a simple RC network? If so, what is the time constant?

End of Part 1 — Go Home and Reflect

Part 2

E. Activation and Inactivation of Na^+ and K^+ Currents

Current through an open channel changes with voltage. Moreover, the number of open channels changes with time and with voltage. Hodgkin and Huxley showed that a quantitative description of the time dependence and voltage dependence was sufficient to explain the action potential. To describe the time and voltage dependencies, Hodgkin and Huxley wrote formal, mathematical functions of the variables that could be made congruent to the conductances experimentally measured. The next logical step in understanding the response is to understand these functions: the state variables m, n and h.

n corresponds to Hodgkin and Huxley's parameter n^4 which represents the fraction of activated K^+ channels. n is a function of time and voltage. As the membrane is depolarized, the percentage

of K^+ channels that are open increases. In the squid axon, the K^+ channel does not inactivate, so a high value of n implies a high K^+ conductance.

h corresponds to the instantaneous proportion of sodium channels that are available to be opened. It is often referred to as sodium inactivation or simply, inactivation. At a steady state, h approaches 1 when V_m is held 5 to 10 mV below the resting potential. This corresponds to the case where virtually none of the sodium channels are inactive, i.e. in a state where a depolarizing pulse can open them.

If V_m remains depolarized by 40 mV or more above resting potential, h approaches 0, and all of the sodium channels are inactive. Therefore, depolarizing pulses added to a 40 mV steady depolarization do not open sodium channels, and there is no inflow of Na^+.

m corresponds to the m^3 parameter which gives the proportion of openable Na^+ channels that are actually conducting Na^+ across the membrane. Thus, the product of m^3 and h gives the proportion of the fixed population of Na channels that are conducting Na^+ ions, as a function of V_m and time. Hodgkin and Huxley used m and h to give Na^+ current, I_{Na}, as a function of maximum sodium conductance, $\overline{g}_{Na}$, and the driving force, $V_m - E_{Na}$, where m^3h contains the voltage and time dependent opening and inactivation of the Na channels.

Their equation for I_{Na} is:

$$I_{Na} = m^3 h \overline{g}_{Na}(V_m - E_{Na})$$

analogously for potassium and leakage currents:

$$I_K = n^4 \overline{g}_K(V_m - E_K) \text{ and } I_L = \overline{g}_L(V_m - E_L)$$

Look at the values of m, n and h as the action potential progresses by exposing the state plot form.

1. Why are the initial values of m, n, and h not zero at the beginning of the trace? (expose the **State Plot** to see m, n and h)

2. Describe qualitatively the changes in proportion of open Na^+ and K^+ channels during the course of the action potential.

F. Voltage Clamp

In this experiment you will redo the initial voltage clamp procedure Hodgkin and Huxley (H-H) performed to characterize the sodium and potassium currents. Toggle to the voltage clamp mode.

1. For different clamping voltages, determine the maximum Na^+ conductance. Plot this as a function of clamp voltage.

2. At what voltage do the channels start to activate strongly?

3. Repeat for K^+.

4. The next exercise is to go through some of the same laborious calculations as H-H and determine the rate constants for the channels. For a clamp potential of 20 mV, determine α and β values for m, n and h graphically.

5. Does V_m always equal the command (clamp) voltage?

6. What causes the large injected current spikes?

G. Current and Conductances

Return to current clamp mode and observe an action potential (pulse width: 1 ms, amplitude: 0.1 μA).

1. Why does the action potential stop rising and start to decrease?

2. Why is there an initial sharp peak in the sodium current?

3. What happens to the instantaneous total of ionic currents during an action potential?

With no stimulus, the sodium and potassium currents are balanced by the *leak* current to achieve a constant resting level of the transmembrane voltage. This leak pathway is modelled as a battery in series with a static leakage resistance. See figure 1.

4. What physical processes are modelled by the leakage pathway?

5. By blocking the Na^+ and K^+ channels, determine the leakage voltage.

6. By unblocking the channels and balancing the currents at rest, what is the leakage resistance.

H. Accommodation

A stimulus of low amplitude and long duration might be expected to eventually elicit a response. Instead, it can actually reduce the excitability of the membrane. This property of adapting to persisting stimuli is called accomodation.
Set the following parameters:

Base Current Level:	0.0 μA
Pulse 1 Current:	0.07 μA
Onset Delay 1:	40 msec
Pulse 1 Width:	1 msec
Pulse 2 Current	0.0 μA

Observe the response of the membrane to the current pulse. Now set the *Base Level Current* to 0.03 μA and now observe the membrane's reaction.

1. Using m, n and h to help you, why does the pulse that is normally above threshold fail to produce an action potential?

2. What is a plausible physiological occurrence of such accommodation?

3. Set the base current level to 0 μA. Choose a $-0.1\mu A$ stimulating pulse of 5 ms duration and observe the plot. What happens when the stimulus is turned off and why?

I. Oscillatory Behaviour

Nerve cells may be induced to fire rhythmically by either increase of extracellular potassium concentration or depolarization of the membrane by electrical current pulses. Note that these stimuli are not periodic yet produce rhythmic behaviour.

During experiments, *in vitro* conditions are not the same as those *in vivo*. The bath will not be the exact same as the living, active environment inside the frog. Ions may accumulate intra- and extracellularly as both are of finite space and the nerve cell may not be given time to return to initial conditions between experiments.

By changing the external concentration of an ion, one changes the Nernst potentials for that ion and will affect the cell's susceptibility to fire an action potential.

1. With no current injection, change the level of $[K^+]_e$ and produce plots of the relationship between $[K^+]_e$ and the following:
   (a) firing frequency,
   (b) resting level,
   (c) amplitude.
2. Reset the concentrations to their default levels and set the following parameters: base current = 0 μA; simulation time = 100 msec; pulse 1 width = 100 msec; onset delay 1 = 0 msec. Starting at a pulse 1 current of 0 μA produce plots of stimulus strength versus the following:
   (a) firing frequency,
   (b) resting level,
   (c) amplitude.
3. Compare the effects of the above two stimuli taking care to explain any similarities and differences.
4. How do these frequency curves relate to the refractory and latency properties (from part 1)?

J. Animal Experimention

1. Should animal experimentation be banned?restricted? Explain your reasons.
2. If an appropriate computer model is available, would it be a viable alternative for animal experimentation? Explain.
3. A marketing research question: Given a live frog on which to experiment, would you
   (a) Whisk the frog away in your bag and set it free near a stream?
   (b) Snap its neck, decapitate it, pithe the head and cheerfully proceed to experiment?
   (c) Give the frog to the overworked T.A. to prepare?