Name: Sign:

Exercise 3I:
Neural models I: the axon

1 Objectives

In this exercise you will study a piece of a cable, representing first an unmyelinated and then a myelinated axon. In the voluntary exercises you may study an active dendrite if you wish. When you are finished you should:

- have some hands on experience in running neuron simulations using the GENESIS simulator
- understand the differences between active and passive cables
- understand current clamp and voltage clamp measurements to study ion channels and their reversal potentials
- understand action potential production and propagation
- have answered the questions wherever you see the label $->$

2 Part I: A homogeneous axon

The first part of the lab is to study the features of a piece of a homogeneous axon. The files are in the directory called "squid". Start the simulation by typing "genesis Squid" in a terminal window. The model has already been initialized with some reasonable parameter values, so all you have to do to run the simulation is:

click on the "RESET" button in the "Simulation Control" panel
click on the "RUN" button in the "Simulation Control" panel

If you haven't studied the manual page added last in this lab instruction, look at the on-line help by clicking at the "HELP" button in the popups window. The simulation is set to perform a current clamp simulation. This is indicated by the presence of a window entitled "Current Clamp Mode". During the simulation the electrode will produce a constant injection current into the membrane and the resulting potential will be measured.

The simulation which you just observed was for a constant current pulse, which is shown in the lower left graph panel. The upper left graph shows the membrane potential. You can see that 3 action potentials were generated during this 50 msec simulation. Now let's change the injection current and see

what happens. At the bottom of the screen there is a control panel labeled ”Current Clamp Mode” and below is a panel with seven dialog-buttons. They have both the properties of a dialog box, which accepts input from the keyboard (right), and a button with a text label (left) that executes a command script when it is pressed. Let’s change the peak injection current from 0.1 to 1.0.

position the cursor to the right of the last digit in the ”Pulse 1 Current” field.
type $< DELETE >$ to backspace over the 0.1 and then type 1.0 the value in the dialog field should now display 1.0

At this point we need to activate the dialog button to send this new value to the simulator. There are two ways to do this; you can either hit $< RETURN >$ when the mouse is within the dialog field or you can click on the button with the left-mouse button.

If this successfully activated the button, you should see a message appear in the terminal window. The message should say something like ”Setting /pulsegen level1 1”. If you forget this the simulator will not know about the changes you make to the dialog!

Now we’ll run the simulation again.

click the ”RESET” button on the control window
click the ”RUN” button on the control window

There should be a dramatic change in the time-course of the membrane potential at this higher current injection level. Experiment with the injection level to see what happens to the axon at higher injection currents. Also try decreasing the injection level until an action potential is no longer elicited by the stimulus.

2.1 More current clamping

Looking in the ”Squid Axon” window at the ”channel current” plot you may notice that the curve for the Na-current displays a bimodal peak during an action potential, explain why? Hint: Read the section on state plots below. Consider the membrane potential, the activation m and inactivation h, and the reversal potential.

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To study the absolute refractory time of the action potential, you may experiment with trains of short pulses. In the ”Current Clamp Mode” window, click on the ”Single Pulse” toggle so that it reads ”Pulse Train”. Set the stimulation duration to 1 msec. Now vary the delay between pulses. What is the minimum

interval between action potentials that you can achieve?

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2.2 State Plots

One can learn a great deal by studying plots in which one of the Hodgkin-Huxley channel activation parameters (the K activation ”n”, the Na activation ”m”, or the Na inactivation ”h”) is plotted as a function of the membrane potential, V.

In order to view such a plot, click the left mouse button on the ”toggle” button labeled ”State Plot Hidden”. The label will change to ”State Plot Visible”, and a state plot graph will appear. Clicking the button again will hide the graph. The default plot is to show the K channel ”n” parameter on the y-axis and the membrane potential on the x-axis. The dialog boxes at the bottom of the graph allow you to change these defaults.

2.3 Voltage Clamping

You can also perform voltage clamp experiments in this simulation.

click on the ”Toggle Vclamp/Iclamp Mode” button at the bottom of the control window.

You should notice several things changing on the screen. All the changes are being controlled by the script attached to the ”Toggle” button. The integration time step has been reduced from 0.1 msec to 0.01 msec, and a new ”Voltage Clamp Mode” window has appeared in place of the ”Current Clamp Mode” window. Again, reasonable default values have been selected.

Perform a voltage clamp experiment to estimate the sodium reversal potential. You can read about measuring reversal potentials in JW 6.2 (see specifically figure 6.5). Hint: Simplify the work by blocking the K current. As a simulation has no noise, you can use the steady-state values (which have lower amplitudes but are more well defined) instead of the instantaneous.

What is the estimated reversal potential?

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Why did you block the K current?

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2.4 Changing Extracellular Concentrations

You can alter the extracellular concentrations of the ions through the ”External Concentration” window”. Any changes in concentration will cause the reversal potentials to be re-calculated. To reset the values back to the initial ones, just click on the ”Default Values” button.

In Current Clamp mode, set the injection current (Pulse 1 Current) to zero. Now increase the external potassium concentration [K] until spontaneous oscillations occur. Why does the increased level of [K] cause oscillations? Do you expect similar results to occur when [Na] is varied?

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2.5 Channel Blocking

In order to explore the effects of blocking one of the channels, you may click on one of the toggle buttons for blocking and unblocking a channel. When a channel is blocked, mimicking the effect of TTX on the Na-channel and TEA on the K-channel, its conductance is set to zero. (Activation parameters for a blocked channel are still calculated and may be plotted, but are relatively meaningless because they will have no effect on the membrane potential.)

What are the main effects as seen in current clamp and voltage clamp for blocking the Na channel?

$->$

What are the main effects as seen in current clamp and voltage clamp for blocking the K channel?

$->$

2.6 Inactivation of the Na channel

The Na channel has an inactivation which is voltage and time dependent. Add a Genesis script by typing ”slow_ramp” in the terminal window. That will load the file slow_ramp.g into Genesis. (If you want to see how the script looks like, type ”cat slow_ramp.g”.) Now, execute the function do_ramp by typing ”do_ramp” in the terminal window. The script will make a 100 ms simulation with a ramp current ending at 0.1 μ A. That current earlier produced an action potential, but now it failed, why?

$->$

3 Part II: An inhomogeneous axon

For the second part of the lab you will study the characteristics of a piece of a myelinated (inhomogeneous) axon using several compartments for the axon. First you have to quit the Genesis simulation. The files are in the directory called “cable”. Start the compartmental version by typing ”genesis Cable”. You will get a new set of windows that work pretty much like the previous ones. If you would like to learn about the specifics of this simulation, click on the ”HELP” button in the control window.

First you need a few steps to set up your simulation. In the control window, click on the ”Change Cell Parameters” button to get that window up. Next, in the ”Cable Compts.” field, type 50 to add 50 serially connected axonal compartments to your soma. Then click on the ”Add/Remove Plots” button to get that window visible. In the ”Add/Remove Plots” window, add the compartments you would like to see plotted in the graph window called ”somagraphs” by giving the axon compartment number in the ”Plot Compt. #” field.

The axon you got now is passive. To perform a simulation click on ”RESET” and then ”STEP” in the ”control” window.

To add Na and K channels to the soma and axon, include a Genesis script by typing ”active” in the terminal window running the Genesis interpreter. Then initialize the simulation by typing ”init” in the terminal window. Perform a simulation to test the homogeneous active axon. Now, to produce an axon with a pattern of one node of Ranvier and N-1 internodes, type ”myel N” in the terminal window. (For example, if you type “myel 10” you will get 1 active node of Ranvier followed by 9 passive internodes followed by 1 node of Ranvier etc. repeating along your axon.)

What number of compartments (including the node of Ranvier) maintains a constant amplitude of the action potential?

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What parameters influence this number? Hint: See JW ch 7.3.

− >

What is the propagation velocity (compartments / sec)?

− >

What parameters influence the velocity?

− >

The axon is homogeneous, so the action potential could travel in any direction. Why does it only travel from soma and outward, and not backwards when a node of Ranvier is activated?

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In Multiple Sclerosis the myelinization is ruined when the glia cells are damaged by the immune system. Confirm the effect of the action potential amplitude and velocity by changing Rm.

How much did you change Rm and how much was amplitude and velocity affected? Hint: You can alter Rm in the "Cell Parameters" window labeled param_menu. Alternatively you can use a function in the file "active.g" called "changeRm" that takes a parameter Rm.

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4 Voluntary exercise on active dendrites

This set-up could also be used to study a soma with one active (thin) dendrite. First, reset any changes you may have done by yourself and remove the internodes of your axon.

1) In the "Change Current Injection" (and "Change Synaptic Input") window you can set the compartment where the current injection/ synaptic input goes. Study the difference between the potential amplitude propagation from the soma going outwardly and from the tip of the dendrite going inwardly with and without Na and K currents. Hint: You can set the conductance to zero by using the functions changeNaCond, changeKCond, setNaCond or setKCond.

2) By replacing the single input current stimulation with a train of inputs, you can study how the attenuation of inputs depends on frequency and on injection site (soma or distal dendrite). Hint: You can perform a number of steps in the simulation by "step T -time", where T is in milli seconds, and then alter the current injection amplitude and then do some more steps etc. Alternative, look how a train of inputs was generated in the "Squid" lab.

3) Make a function yourself that changes the compartment diameter of the dendrite from 50 μm (the same as the soma) for the compartment attached to

the soma, down to 1 μm diameter at the end. Now, compare the difference between inward and outward propagation. Hint: Look in the file active.g to see how compartments are modified.

4) By adding a new function derived from myel you can add hot spots (active regions) to your dendrite. Make most of the dendrite passive or weakly active and add hot spots on top of this. Study how synaptic input is attenuated with and without hot spots.

5 Documentation for the Genesis Squid lab interface

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                                SQUID

An Introductory Tutorial for GENESIS, XODUS, and the HODGKIN-HUXLEY model

By M. Nelson, Caltech, April 1989 - Modified by D. Beeman, June 1991, Dec 1994
                                  - Modified by E. Vigmond, September 1993
                                  - Adapted for biomod by Erik Fransen, 2002


Running the Simulation
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it is a panel with seven dialog-buttons. They have both the
properties of a dialog box, which accepts input from the keyboard
(right), and a button with a text label (left) that executes a command
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script when it is pressed. Let's change the peak injection current
from 0.1 to 1.0.

position the cursor to the right of the last digit in the "Pulse 1
Current" field.
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currents. Also try decreasing the injection level until an action
potential is no longer elicited by the stimulus.

Controlling Graphs
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If the graph of the clamp current (lower left panel) has gone off scale,
rescale the graph by either of two things:

point with the cursor on the largest number on the axis scale, left-mouse
klick and drag "out" to increase range or "in" to decrease range.

or, click on the "scale" button in the upper left corner of the graph

A window containing dialog buttons for the coordinate range should appear.
Adjust ymin and ymax to bring the graph into range. Remember to activate
the dialog buttons by either hitting <RETURN> in the dialog field or by
clicking on the button after you've made your changes. The x-axes of all
graphs are automatically scaled to the time of the simulation.

click on the "DONE" button to hide the "Graph Scale" window

It is often convenient to leave the "Graph Scale" window visible if you are going to be making alot of scale changes. Just drag the window to a convenient place on the screen and leave it there.

The "Graph Scale" window also has an "overlay" dialog button.
When overlay = 1, any existing plots in the graph will be held and new simulation results will be overlayed. Try this now.

Set overlay = 1 for the graph of membrane voltage and run the simulation at several values of the clamp voltage (remember to RESET between runs). To clear all but the last graph, click on the "Clear Graphs" button.

Printing Graphs

You can get a printout of a graph by positioning your cursor withing the graph area and typing <CTRL-p>. There is no user feed-back so type only once and check the printer queue! An even better way to capture a portion of the screen is to use a screen-grabbing utility like "xv" or "xwd" from your favorite X-windows archive site.

Units used in the simulation

```
Units:  time            msec
        length          micro m
        potential       mV
        conductance     mS (mmho)
        resistance      k ohm
        capacitance     micro F

        specific axial resistance       k ohm-cm
        specific membrane conductance   mS/cm^2
        specific membrance capacitance  micro F/cm^2
```

Windows

Widget color code

```
green       headline
yellow      input field and button
```

```
dark blue    toggle button off
red          toggle button on
light blue   command button
```

```
window name headline               content

control     Simulation control      main simulation parameters and controls
graphs      Squid Axon              membrane, electrode and channel states
phase_plot                          channel gate plots
popups                              help, phase plot on/off, Na and K block
iclamp      Current Clamp Mode      current electrode parameters
vclamp      Voltage Clamp Mode      voltage electrode parameters
exconcen    External Concentrations ringer bath concentrations
```

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control "Simulation control"
  RESET reset parameters to initial conditions and recalculate state
        variables will clear the graphs if Overlay is off
  RUN   start the simulation
  STOP  stop the simulation
  QUIT  quit the GENESIS simulation
  time  simulation time
  dt    numerical ODE solver time step length
  Toggle Vclamp (voltage clamp) / Iclamp (current clamp) Mode
  Overlay of graphs on/off
  Clear Graphs

iclamp "Current Clamp Mode"
  Base Current Level  current level outside Pulse 1 and Pulse 2
  Pulse 1 Current     current level during Pulse 1 (first pulse)
  Onset Delay 1       time before start of Pulse 1
  Pulse 1 Width       duration of Pulse 1
  Pulse 2 Current     current level during Pulse 2 (second pulse)
  Onset Delay 2       time before start of Pulse 2
  Pulse 2 Width       duration of Pulse 2
  Single Pulse/Pulse Train one or several pulses

vclamp "Voltage Clamp Mode"
  Holding Voltage       start (first) voltage level
  Holding Time          start voltage duration
  Pre-pulse Voltage     middle (second) voltage level
  Pre-pulse Time        middle voltage duration
  Clamp Voltage         final (third) voltage level
  Clamp Time            final voltage duration
```

```
graphs "Squid Axon"
  axon membrane potential graph
    red measured membrane potential
    blue injected potential

  electrode injection current/potential
    red injected current
    blue measured membrane current

  channel conductance
    red K conductance
    blue Na conductance

  channel current
    red K channel current
    blue Na channel current

popups
  HELP on-line help
  toggle "State Plot" on/off
  toggle Na channel blocker (TTX) added to bath on/off
  toggle K channel blocker (TEA) added to bath on/off

phase_plot
  "State Plot"
    phase plotting of n, m, h or V versus n, m, h or V
    select state variable to plot on vertical (y-axis) and horizontal
      (x-axis) respectively
  "H-H activation parameters vs time
    plots gate states m (black), h (red) and n (blue) versus time

exconcen "External Concentrations"
  K concentration in ringer solution
  Na concentration in ringer solution
  reset to default values


Genesis interpreter
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If you would like to test the Genesis interpreter, get your terminal
window visible. The simulation objects are arranged pretty much like a
directory tree. Type "ce /" to go to the root of the object hierarchy.
Type "le" to get a directory list. Go to the axon by typing "ce axon".
Here you see a graph object associated with the main graph window, a
Na channel and a K channel. To see all the parameters of the axon,
```

type "showfield -a". Information between objects are passed with messages. Type "showmsg ." to see the messages of the axon. The axon is a compartment object, to see the man page of the compartment object, type "help compartment". Executing a new script file is done by typing the name of the file at the Genesis prompt, ie "slow_ramp". That will load the file slow_ramp.g into Genesis. Now, execute the function do_ramp by typing "do_ramp" in the terminal window. If you want to load a new version of a function, just load the file into Genesis again.