GENERATION OF ACTION POTENTIALS

INTRODUCTION:

The Hodgkin-Huxley model of action potential generation in the giant axon of the squid forms the basis of most models used in computational neuroscience. In this lab, you will work with the Hodgkin-Huxley model to investigate the typical neuronal behaviour. The simulations will be carried out 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 phenomena as:

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

Specific instructions for the lab report are designated with the  symbol.

GENERAL OVERVIEW:

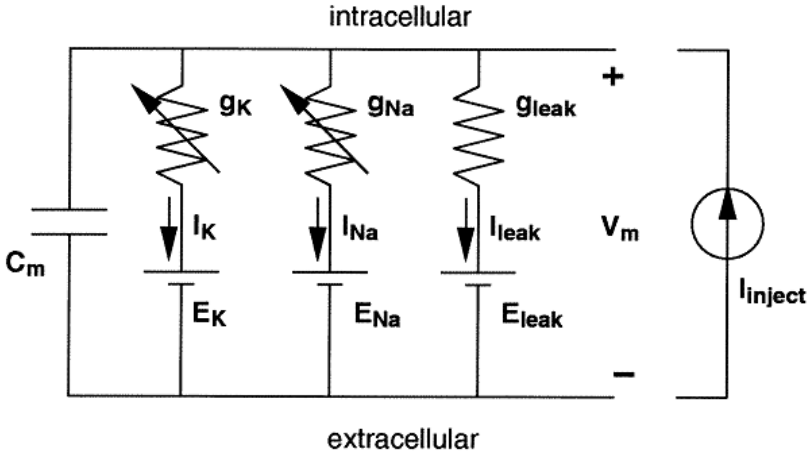

Figure 1: Equivalent circuit of the parallel conductance model of cellular membrane. Note the voltage and current conventions.

In Figure 1 you can see the equivalent circuit of the patch of membrane. Several conventions were used by Hodgkin and Huxley in their models. First, the entry of cations into the cell (e.g. Na^+ entry) was 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. Second, the resting membrane voltage was established to be 0 mV.

Modern convention sets the resting membrane voltage at around -70 mV, which we will be using for this lab.

Ionic equilibrium potential:
The equilibrium potential for ion C is given by Nernst equation:

$$E_C = \frac{RT}{zF} ln\left(\frac{[C]_{out}}{[C]_{in}}\right)$$

where R is the ideal gas constant (8.3145 J/mol K),
T is absolute temperature,
z is the valence of the ion,
F is Faraday's constant 96480 C/mol,
$[C]_{out}$ is concentration of C outside the membrane,
$[C]_{in}$ is concentration of C inside the membrane.

Voltage channels:

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.*

- The parameter *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.
- The parameter *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 5-10mV 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 40mV or more above resting potential, *h* approaches 0, and all of the sodium channels are inactive. Therefore, depolarizing pulses added to a 40mV steady depolarization do not open sodium channels, and there is no inflow of Na^+.
- The parameter *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.

K^+ channel:
The current through K^+ channel in squid giant axon is given by:

$$I_K = \bar{g}_K * n^4 * (V - E_K)$$

where $\bar{g}_K$ is the peak conductance of K^+ channel, *n* is the fraction of activation gates open. The transition from closed to open state has first order kinetics:

$$\frac{dn}{dt} = \alpha_n * (1 - n) - \beta_n * n$$

where α_n and β_n are transition rate constant (shut-to-open and open-to-shut respectively), and can be found as:

$$\alpha_n = \frac{n_\infty}{\tau}$$

$$\beta_n = \frac{1 - n_\infty}{\tau}$$

Na^+ channel:
The current through Na^+ channel in squid giant axon is given by:

$$I_{Na} = \bar{g}_{Na} * m^3 * h * (V - E_{Na})$$

where $\bar{g}_{Na}$ is the peak conductance of Na^+ channel, *m* is the fraction of activation gates open and *h* is the fraction of deactivation gates open. The transitions from closed to open states for *m* and *h* follow the equations described in "**K^+ channel**" section.

Membrane Current:
The current through the membrane is given by:

$$I_m = C_m\, dV/dt + I_K + I_{Na} + I_L$$
$$= C_m\, dV/dt + \bar{g}_K * n^4 * (V - E_K) + \bar{g}_{Na} * m^3 * h * (V - E_{Na}) + g_L * (V - E_L)$$

where g_L is the leak current and E_L is the leak reversal potential.

GETTING STARTED:

In order to launch the software used in this lab,

If you are on Linux: open the Terminal and run "*/n/share/copy/ece445f/squid_demo*" (without quotation marks).

If you are on Windows: download the lab3_files.zip package. Open Anaconda PowerShell Terminal and run

1- *"cd [file path]"*

2- *"python3 squid_demo.py"*

This will launch the GUI you see in Figure 2. If it is your first time running the executable it can take several seconds to build up font cache before the GUI appears.

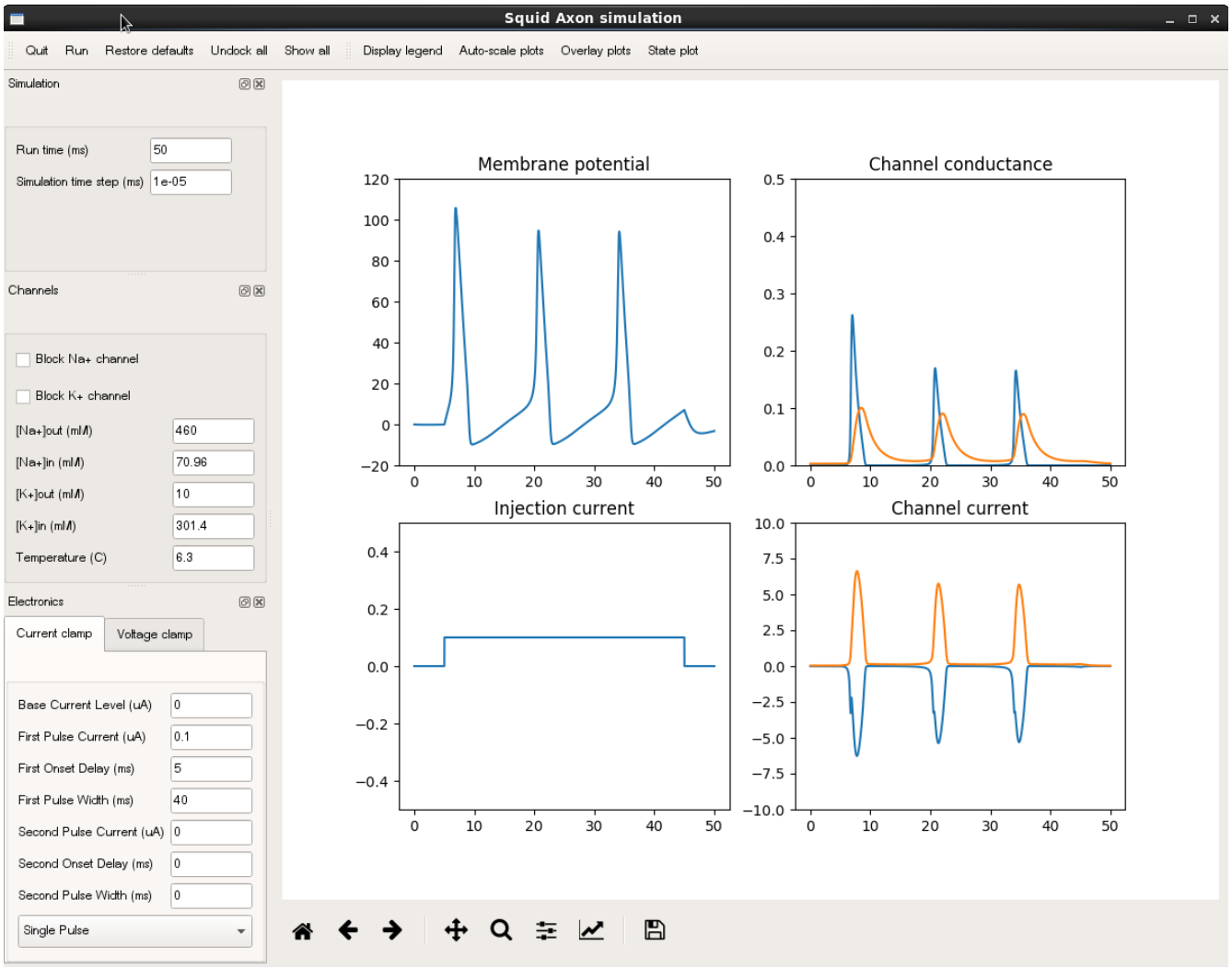

Figure 2: Lab 3 user interface

Click on “Run” to see the results in Figure 2. Click on State plot to see plots of state variables shown in Figure 3.

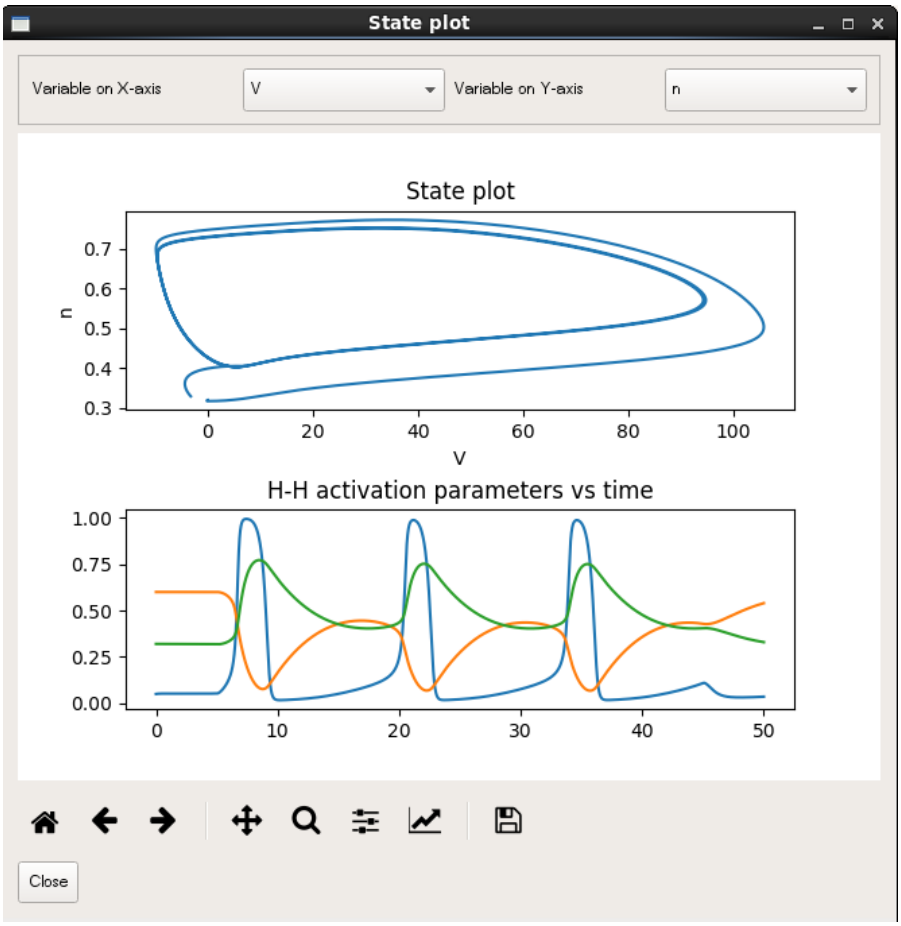

Figure 3: State plots

Please see APPENDIX for additional information on GUI and plotting help.

EXPERIMENTS:

A. Excitation

Set the *First Pulse Width* to 1ms (keeping everything else as default) and observe the generation of an action potential.

Describe the action potential: including characteristics like threshold potential, maximum amplitude, and hyperpolarization.

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.

Find the minimum current pulse amplitude (*First Pulse Current*) which causes the firing of an action potential (excitation threshold) for various pulse widths and **plot** the results as

a **Strength-Duration Curve**.

Pulse width (ms)	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	2.0
Current amplitude (μA)									

1. Is there a simple relationship between stimulus duration and strength?
2. The *rheobase* is the maximum amplitude which cannot trigger an action potential regardless of duration. From your graph, what is its magnitude?

B. Latency

Produce a point-by-point **Latency Curve** for the membrane by applying current pulses having a pulse width of 1ms and various pulse amplitudes = [0.06, 0.07, 0.08, 0.1, 0.12, 0.14, 0.16…] μA and measuring the time between the stimulus onset and the time of the peak of the action potential. **Plot** the results.

C. Refractoriness

Nonlinear oscillators exhibit regions in their cycle where 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.

Apply a first pulse of lμA magnitude and 1ms duration to the squid neuron. By application of a second pulse, of 0.3 ms duration, at a set delay after the first pulse, try to elicit an action potential. In such a manner, **plot** a graph of the minimum pulse 2 amplitude required to produce a second action potential for various delays.

1. How long is the **absolute** refractory period?
2. How long is the **relative** refractory period?

Hint: certain pulse 2 amplitudes will "drive" the circuit regardless of how short the delay is after the first pulse. Think about how you would distinguish non-linear phenomena (action potential generation) from the artefact produced by a very large pulse.

D. Passive Properties

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

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?

E. Na^+ and K^+ Conductance

Setup a single current pulse with width of 1ms and amplitude of 0.1 μA. Run the model and look at the values of *m*, *n* and *h* as the action potential progresses by clicking on the State plot

- 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*.)
- Describe qualitatively the changes in proportion of open Na^+ and K^+ channels during the course of the action potential.
- What is the **time** difference between:
    1. Peak g_{Na} and the peak of the action potential?
    2. Peak g_K and the peak of the action potential?

  Note: plots show x-y coordinates of the cursor, however you might have to undock control panels if your screen is not large enough (see APPENDIX)

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.

NOTE: Holding Voltage and Holding Time refer to the initial baseline values, you should only change Clamp Voltage/Clamp Time.

For different clamping voltages = [-65, -60, -50, -40, -30, -0, 50, 100, 200 ... 500] mV, determine the maximum Na^+ conductance. **Plot** g_{Na} as a function of clamp voltage. At what voltage do the channels start to activate strongly?
Repeat for K^+, i.e. **plot** g_K vs clamp voltage = [-65, -60, -50, -40, -30, -0, 50, 100, 200 ... 500] mV.

1. Here we follow the same calculation steps as H-H performed to determine the rate constants for the channels. For a clamp potential of -50 mV, graphically determine (using the STATE MODE PLOT) α and β values for *m*, *n* and *h*:

   α_m = _____ β_m = _____

   α_n = _____ β_n = _____

   α_h = _____ β_h = _____

2. What causes the large injected current spikes?

G. Current Clamp and Conductances

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

1. Why does the action potential stop rising and start to decrease?
2. Describe the time course of the total ionic current during an AP. Why is there an initial sharp peak in the sodium current? What happens to the instantaneous total of ionic currents during an action potential? (Hint: Think back to part **E**)

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.

2. By blocking the Na^+ and K^+ channels, determine the leakage voltage. By unblocking the channels and balancing the currents without stimulus determine the leakage resistance. What are the specific values of E_{leak}, g_{leak}? (Hint: Think back to membrane current equation)
3. What physical processes are modelled by the leakage pathway?

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 accommodation. Set the following parameters:

Base Current Level:	0.0μA
First Pulse Current:	0.07μA
First Onset Delay:	40ms
First Pulse Width:	1ms
Second Pulse Current:	0.0μA

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

1. Describe the change in V_m as the Base Level Current is changed from 0.0μA to 0.03μA

2. Using *m*, *n* and *h* to help you, why does the pulse that is normally above threshold fail to produce an action potential? What is a plausible physiological occurrence of such accommodation?
3. Set the base current level to 0μA. Choose a -0.1μA stimulating pulse of 5ms 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 squid. 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.

A. With no current injection, for $[K^+]_{out}$ = [15, 16, 17, 18, 20, 22, 24] mM, **plot** the relationship between $[K^+]_{out}$ and the following:

1. AP firing frequency
2. AP resting level
3. AP amplitude

Reset the concentrations to their default levels and set the following parameters:

Base Current Level:	0.0μA
Simulation time:	100ms
First Pulse Width:	100ms
First Onset Delay:	0.0ms

B. For first pulse current amplitude = [0, 0.1 … 1.2] μA, **plot** stimulus strength versus the following:

1. AP firing frequency,
2. AP resting level,
3. AP amplitude.

Compare the effects of the above two stimuli (increased $[K^+]_{out}$ and increased *First Pulse Current*), taking care to explain any similarities and differences. How do these frequency curves relate to the refractory & latency properties (from Parts **B** and **C**)? If you notice any differences between amplitudes of AP, are they what you would expect? Why or why not?

C. Keeping everything at default values (and with no current injection), vary individually $[K^+]_{in}$, $[Na^+]_{out}$, and $[K^+]_{in}$. Which ion has more influence on cell's ability to fire an action potential?

While Hodgkin and Huxley worked on a squid axon, their model has been used for other conditions, including human neurons. However human neurons have vastly different ionic concentrations and temperature than the squid axon, so care must be taken when results are compared across species.
Set the standard current injection (pulse width 1ms, amplitude 0.1μA). Run the model with the default channel parameters, then change the parameters as follows:

[Na+]out:	145 mM
[Na+]in:	10 mM
[K+]out:	3 mM
[K+]in:	130 mM
Temperature:	37° C

Compare the two action potentials (Note: it could be helpful to use the Overlay functionality)

J. Final Thoughts

1. Hodgkin-Huxley model and models like it have several assumptions inherent in their design. List at least two assumptions. What could be the potential limitations of the Hodgkin-Huxley model?
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?

Lab Write-up Format

title/date	Laboratory title and date the experiment was performed
name	Names of **everyone** in the laboratory group [one **(1)** lab report is to be submitted by each lab group]. Include full names and all student numbers.
purpose	One or two sentences identifying the objective or purpose of the investigation
results/discussion	All questions from the lab investigation and write-up section should be answered here. Any additional observations, analysis performed during the laboratory may be included. In addition, a simple one paragraph explanation of what you did and what you found out should be included at the end of the observations/discussion section. Write this paragraph as if you were explaining your results to someone who is not familiar with the laboratory topic (i.e. someone who hasn't taken this course before). **(maximum of 200 words for this paragraph).**
figures	Print out and include all figures (All figures requested in the lab investigation and write-up section must be included, along with any

additional figures that may complement your observations/results).

APPENDIX:

A1. GUI help:

In order to increase the size of plots and save screen space, you can undock the control panels. To undock all panels in one go, click the "Undock all" button in the tool bar. An undocked panel will be always on top of the main window. If this is a problem, you can close it by clicking the close button. You can make it visible again by clicking "Show all" button on the tool bar.

You can see the plotting navigation toolbar at the bottom of the plots. While you can hover the mouse cursor on any icon to get a tool-tip, the summary is as follows:

Zoom:

1. Zoom-in:
    a. Zoom-in both X and Y axes:

       Click on the subplot you want to zoom and scroll mouse wheel down, or

       Click magnifier icon and press left mouse button on the subplot and drag. This will zoom the subplot to the rectangle you cover by this. Or

       Click compass icon and press right mouse button on the plot and drag towards top-right.
    b. Zoom-in along X-axis:

       Click compass icon and press right mouse button on the plot and drag towards right.
    c. Zoom-in along Y-axis:

       Click compass icon and press right mouse button on the plot and drag upwards.
2. Zoom-out:
    a. Zoom-out both X and Y axes:

       Click on the subplot you want to zoom and scroll mouse wheel up, or

       Click magnifier icon and press right mouse button on the plot and drag. The whole visible axis area will fit into the rectangle you cover by this. Or

       Click compass icon and press right mouse button on the plot and drag towards bottom-left.
    b. Zoom-out whole plot window along X-axis:

       Click compass icon and press right mouse button on the plot and drag towards left.
    c. Zoom-out Y-axis:

       Click compass icon and press right mouse button on the plot and drag downwards.
3. Go forward/backward in zoom stack: click right/left arrow icon.

Pan:

Click compass-icon and left click-and-drag

Reset to initial plot state:

Click home icon

Change spacing and position of subplots:

Click button with three sliders (this is called the Subplot-Configuration button)

Configure axes:

Click the upwards arrow. In the pop-up dialog select the axis you want to modify and click OK. In the next dialog, you can set the range of X and Y values for the axes and modify several other properties.

Save plot:

Click floppy disk icon

A2. Miscellaneous:

Quantity	Unit
Time	msec
Length	μm (micron)
Potential	mV
Conductance	mS
Resistance	Kohm
Capacitance	uF
Specific Axial Resistance	Kohm-cm
Specific Membrane Conductance	mS/cm^2
Specific Membrane Capacitance	uF/cm^2

Table 1: Units used in simulation

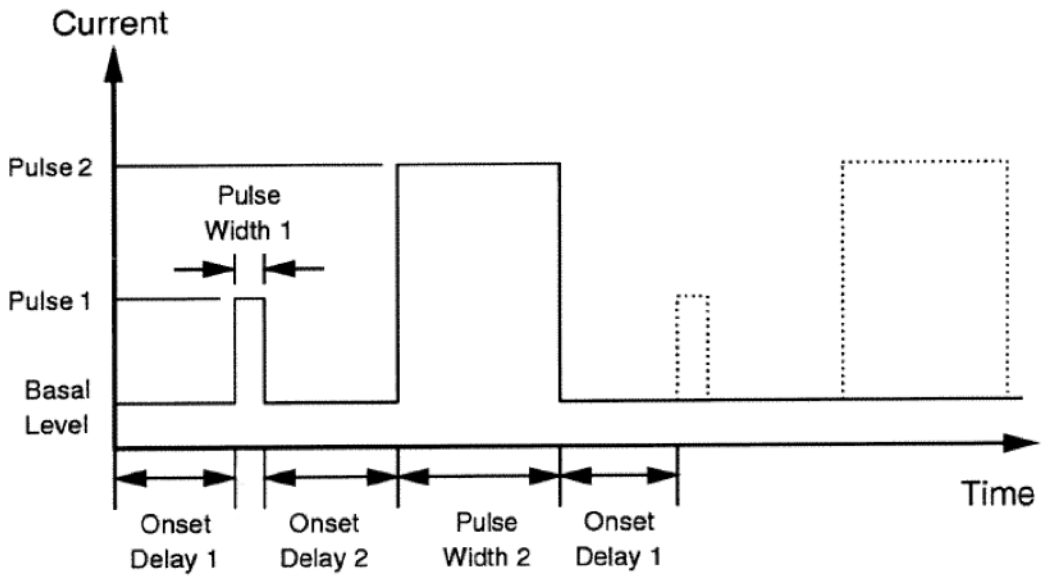

Figure 4: Definitions of quantities defining current clamp stimulus. If a pulse train is specified, the pulses continue *ad infinitum* as indicated by the dashed lines.

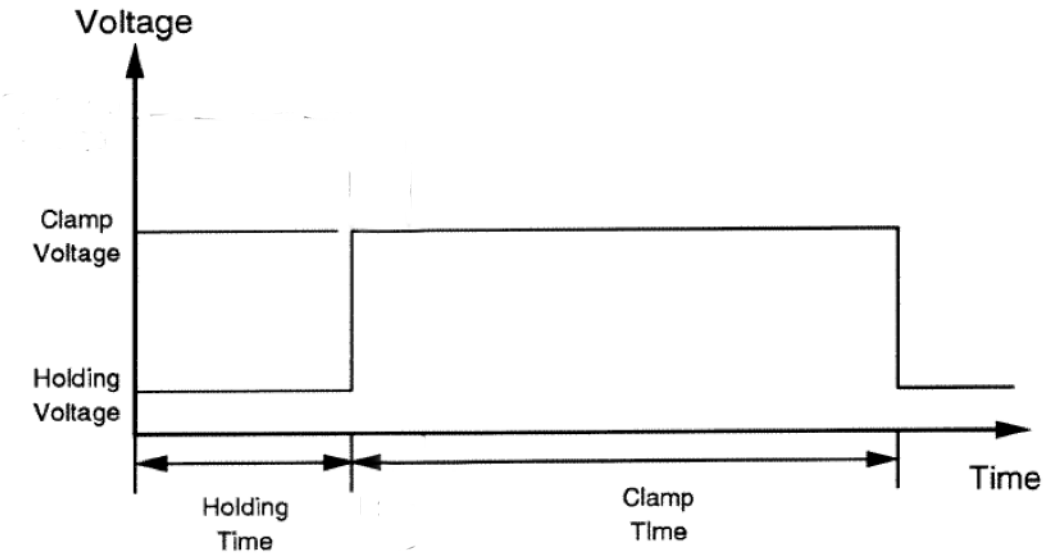

Figure 5: Definitions of quantities defining voltage clamp stimulus.

Note: We would like to thank Subhasis Ray for the original version of both the GUI and plotting summary.