Laboratory 2: Neurosim: Membrane potentials and Action potentials

INTRODUCTION

NeuroSim is a computer program intended for use in teaching neurophysiology, specifically the concepts associated with the resting membrane potential and the action potential. Within the program of NeuroSim is a simulation entitled HH which is based on the equations developed by English physiologists A. L. Hodgkin and A. F. Huxley (J. Physiol. Lond. 117: 500-542, 1952). Much of our current knowledge about the mechanisms of resting membrane potentials and action potentials comes from experiments performed by Hodgkin and Huxley on a giant nerve fiber found in a species of squid called *Loligo forbesi* (adult squid weight 3.5 lb, adult squid length 14 in). Because of the large diameter of this axon (up to 0.8 mm), it became a convenient model for electrophysiological research using small intracellular microelectrodes (tip diameter < 0.5 μm). With two microelectrodes, researchers were able to detect an electrical potential difference between an electrode inserted inside the nerve cell and one placed outside the cell. The internal electrode measured a potential of negative 70 mV with respect to the external electrode. This 70 mV potential difference was called the resting membrane potential. Membrane potentials are therefore expressed as the intracellular potential minus the extracellular potential. A negative value denotes that the cytoplasm is electrically negative relative to the extracellular fluid.

Resting membrane potential

The resting membrane potential is due to specific ions, mainly potassium, and their tendency to diffuse down their concentration gradients until opposed by an electrical force of opposite but equal magnitude. To illustrate, create a model of a membrane by using two chambers (A and B) separated by membrane. Assume the membrane is permeable to cations but not to anions. If two different concentrations of a KCL solution are placed in the chambers (0.1 M KCL in A and a 0.01 M KCL in B), K^+ will begin to diffuse from chamber A to chamber B down its concentration gradient. Initially no electrical potential difference will exist, however, since the membrane is not permeable to Cl^-, side A will begin to accumulate negative charges along the membrane thus becoming electrically negative with respect to side B. The more K^+ that flows from side A to side B, the larger will be the potential difference resulting in an electrical force opposing the diffusion of K^+ across the membrane. The net flow of K^+ will cease when the electrical gradient (inward) equals the concentration gradient (outward). Similarly, all cells have a negative resting membrane potential because of this selective permeability to K^+ through K^+ leak channels (leak channels are channels that are usually open). The magnitude of the electrical potential required to counterbalance the concentration gradient for a given ion can be computed using the Nernst equation (Silverthorn pp 163, 252). The potential energy difference across the membrane can be harnessed by the cell to do work and is the basis of the action potential.

Action Potentials

The action potential represents a rapid change in the membrane potential followed by a rapid return to the resting state. In nerve cells the action potential is the basis of transmitting nerve signals. The action potential is caused by changes in the plasma membrane permeability (conductance) to sodium and potassium ions that is different from the conductance responsible for the resting membrane potential.

At rest the conductance of Na^+ through the membrane is extremely low. However, if the neuron receives an electrical stimulus (of sufficient magnitude) activation gates on voltage sensitive Na^+ channels will open. In contrast to K^+, the concentration of Na^+ is extremely high on the outside relative to the inside of the cell, therefore opening of Na^+ specific channels will cause a rapid influx of Na^+, down both its concentration and electrical gradient causing the charge on the membrane to suddenly become more positive, this is referred to as **depolarization** of the membrane. The membrane potential will increase rapidly in response to the increased positive charge until it approaches the equilibrium potential for the Na^+ ion (E_{Na} = 67 mV) thus converting the potential energy established during the resting membrane potential to actual work. It is important to note that depolarization occurs with minimal changes in the overall concentration of Na^+ or K^+. *(Only one out of every 100,000 Na^+ ions needs to enter the cell to produce a 100 mV change in potential).*

Once activated, the inactivation gates on the voltage sensitive Na^+ channels close after a ~0.5 msec delay resulting in Na^+ channel inactivation (the Na^+ channels have two gates, an activation gate and an inactivation gate, both of which are stimulated by a threshold stimulus). Simultaneously, voltage regulated K^+ channels open, resulting in a net efflux of potassium. This net efflux is in addition to the efflux resulting from K^+ leak channels that are always open. The additional efflux of potassium in combination with the termination of Na^+ influx reverses the initial depolarization and the membrane potential moves back towards the resting potential (**repolarization**) and even beyond (**hyperpolarization**), after which the K^+ voltage channels close and the resting membrane is reestablished (the potassium channels have only one gate which is activated by depolarization and inactivated by repolarization). The small depletions that occur in the K^+ and Na^+ concentrations following each action potential are then reestablished by Na^+/K^+ ATPase. It is important to note that in order for activation of the Na^+ channels to occur there needs to be a stimulus of sufficient strength to exceed the **threshold value**. For example, the threshold value for the squid giant axon is near -55 mV, while the resting membrane potential is near -70 mV. If a stimulus is not sufficient to bring the membrane up to the threshold value (-55 mV) then an action potential cannot be initiated. Although there is an observable potential change at the site of stimulus, it does not result in an action potential and is referred to as a **local or graded potential**. However, if the stimulus is sufficient to reach the threshold value, an action potential is triggered. The action potential differs from the local potential in two important ways: (1) it is all-or-nothing while size of the graded potential depends on the strength of the stimulus and (2) the action potential is propagated down the entire length of the nerve.

Understanding Neurosim

Getting Started:

- To begin this laboratory, go to https://vdi.byui.edu/ and click on the VMware Horizon HTML Access option. Next login using your BYUI student login. Select the BYUI software option, then you should see the windows welcome screen. Depending on your security settings you may need to click on the "advanced options" link and allow this program to run on your computer
- Once you have logged in, you should be able to find the Neurosim 5 shortcut on the virtual desktop. Open the Hodgkin-Huxley model.

Establishing Experiment Conditions:

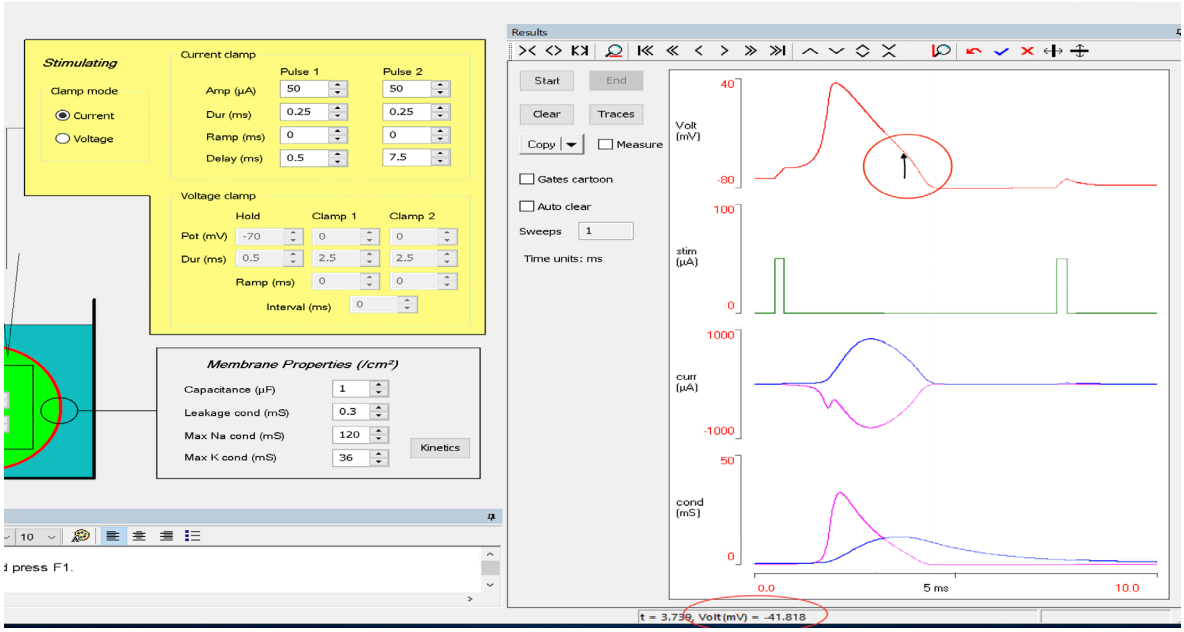
- In order to experimentally manipulate the resting membrane potential, without generating an action potential, we must "turn off" the voltage-activated Na^+ channels. This is accomplished by the addition of the drug **tetrodotoxin** (TTX), which blocks the activation gates on the voltage activated Na^+ channels. The TTX option can be found under the 'drugs' tab.
- **Turn off the pre-set stimulus** by changing the amps (in the current clamp window) to 0.0 for both the first and second pulses.

Recording your results:

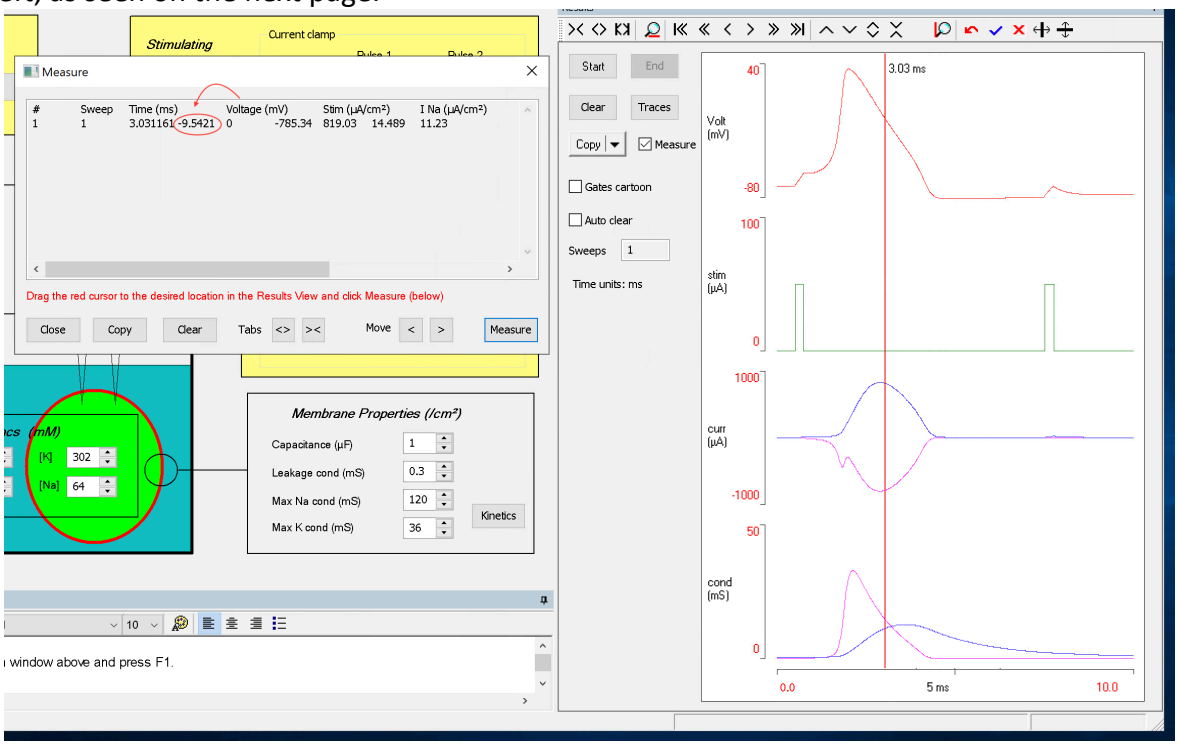
- There are 2 ways you can record your results:
    1. The simplest way is to simply hoover your cursor above the mV tracing you wish to read. (You should see your cursor turn into an arrow). At this point, the reading for that point can be found at the bottom of the window (arrow and results circled in red).

Click on the **Results window** icon on the top of the screen. The results window is currently set up to record several different tracings. Click on the **Traces** button. Notice that each display is generated when an "x" is placed in the box labeled "axis". **Remove the "x" from the stimulus and current boxes** by clicking once on the "x". Finally, **change the X-axis scale** (time) to 100 ms by left clicking on the X axis on the 25 ms label.

Click once on the **Start** button on the Results screen. The observed tracing represents the RMP of the cell. What is the value of the RMP? To measure the RMP, click on the **Measure** button, move the cross hair to any point on the graph, and click once. With the cross hair you can measure voltage (vertical scale) and time (horizontal scale). These data are recorded in a box that will appear on the screen. This is how you collect and interpret data during the lab.



2. Click on the measure button in the results window (sometimes this new tab will open right in front of the graph, and you will need to drag it to a different part of your screen). Once your measure screen is open, you will notice that a red vertical line has appeared. You can click and drag this line to whatever point on the graph you would like. At this point, you will click "measure" in the recently opened window, and it will display your results. (Note that often times it will not display the mV reading under the mV section, it will be located slightly to the left, as seen on the next page.



Changing the axis scale:

- In the event you need to change your x or y axis, go ahead and click on the number at the far end of each axis, and type in the new number you would like. It will then automatically scale your tracings to the new axis.
- You cannot change the axis scale unless the graph has been cleared.

Turning in your lab write up:

- It is not necessary to include any graphs or charts in your lab write up that you submit. Those are for your reference and convenience only. Only the questions for each exercise need to be submitted.

Manipulation of Resting Membrane Potentials

Exercise 1: Increase/decrease extracellular potassium

Based on information from the introduction, there are two factors that help determine the membrane potential of a cell: concentration gradients of ions (mainly potassium) and conductance (or leakiness) of the ion across the membrane. To illustrate how the RMP is determined by these factors, we will manipulate ion concentrations both inside and outside the cell.

Return to the main window. Notice that the green cell has ion concentrations indicated for the extracellular and intracellular environments. For reference, here are those figures;

$[K^+]$ outside:	10 mM	$[K^+]$ inside: 302 mM
$[Na^+]$ outside:	418 mM	$[Na^+]$ inside: 64 mM

1. Be sure that you have the neurotoxin TTX applied for the first 4 exercises. Click the start button to generate a control tracing
2. Alter the extracellular potassium concentration from 10 to 20 mM by clicking on the values and retyping new ones.
3. Continue doing this by increments of 20 until you reach 100 mM.
4. Record your results, and then clear the results tab.

Concentration:	10 mM	20 mM	40 mM	60 mM	80 mM	100 mM
Voltage:						

5. Click the start button to generate a control tracing.
6. Return all concentration values back to setup values.
7. Reduce the extracellular concentrations of K^+ from 10 mM to 5 mM.
8. Record your results:

Concentration:	10 mM	5 mM
Voltage:		

Questions (Exercise 1)

1. What happened to the RMP and why? (Hint: Consider the Nernst Equation)

2. What would be the physiological consequence of increased (hyperkalemia) or decreased (hypokalemia) extracellular K^+ concentrations

Exercise 2. Increase/decrease intracellular Potassium

1. Return all concentration values to default settings, and clear the results tab.
2. Press start to generate a control tracing
3. Raise the intracellular potassium concentration in increments of 50 from 302 mM to 502 mM.
4. Record your results:

Concentration:	302 mM	352 mM	402 mM	452 mM	502 mM
Voltage:					

5. Repeat steps 1 and 2
6. Lower the intracellular potassium concentration in increments of 50 from 302 mM to 102 mM.
7. Record your results:

Concentration:	102 mM	152 mM	202 mM	252 mM	302 mM
Voltage:					

Questions (Exercise 2)

1. Explain the results, what happened to the RMP and why?
2. What had a greater impact on the resting membrane potential, changing intracellular [K^+] or changing the extracellular [K^+]? Why?

Exercise 3. Increase/decrease extracellular sodium:

1. Return all concentration values to default settings and clear the results tab.
2. Click the start button to generate a control tracing
3. Raise the extracellular sodium concentrations incrementally by 10 mM but do not exceed 500.
4. Record your results:

Concentration:	418 mM	430 mM	440 mM	450 mM	460 mM	470 mM	480 mM	490 mM	500 mM
Voltage:									

5. Repeat steps 1 and 2
6. Lower the extracellular sodium concentrations incrementally by 50 mM to 200 mM.
7. Record your results:

Concentration:	200 mM	250 mM	300 mM	350 mM	400 mM
Voltage:					

Questions (Exercise 3)

1. What happened to the RMP and why?
2. How do these changes compare to what happened when you altered extracellular potassium? How do explain the differences?

Exercise 4. Increase /decrease Intracellular sodium:

1. Return all concentration values to default settings and clear the results tab.
2. Click the start button to generate a control tracing.
3. Raise the intracellular sodium concentrations in increments of 100 till you reach 500.
4. Record your results:

Concentration:	64 mM	100 mM	200 mM	300 mM	400 mM	500 mM
Voltage:						

5. Repeat steps 1 and 2
6. Lower the intracellular sodium concentrations in increments of 10 till you reach 4.
7. Record your results:

Cocnentration:	4 mM	14 mM	24 mM	34 mM	44 mM	54 mM
Voltage:						

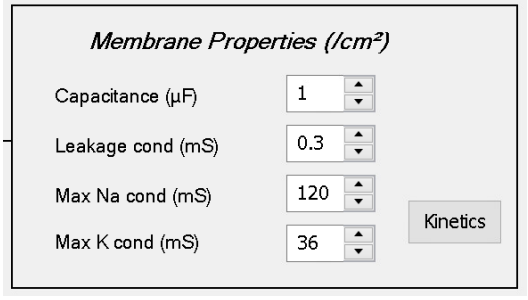
Questions (Exercise 4)

1. Explain the results, how do they differ, why?

Exercise 5. Changes in potassium conductance:

Return all values to default settings and turn off TTX. On the setup window, find the box in the lower right corner that indicates Membrane properties. Capacitance gives a measure of the electrical capacity of the membrane - a greater capacitance means it is harder to generate potential differences when ions are moving (it will have no effect on RMP). The leak conductance gives an indication of overall ionic leakiness, or non-specific ion movement across the membrane. Maximum Na^+ conductance and maximum K^+ conductance indicate the maximum conductance that can occur for each ion. In other words, it is a measure of the number of channels available for each ion including both voltage and leak channels.

For reference, here are the set point values:



1. Make sure the results tab is cleared, then generate a control tracing.
2. Gradually increase the maximum potassium conductance in 20 mS increments until you reach about 150, record the changes in RMP.
3. Record your results:

Conductance:	36mS	50 mS	70 mS	90 mS	110 mS	130 mS	150 mS
Voltage:							

4. Return all conductance values and setting to control levels.
5. Repeat step 1
6. Decrease the maximum K^+ conductance in 10 mS increments until you reach 6, record the changes in RMP.

Conductance:	6 mS	16 mS	26 mS	36 mS	46 mS
Voltage:					

Questions (Exercise 5)

1. What did you observe and why?

Exercise 6. Changes in sodium conductance:

1. Return all conductance values to default settings and clear the results tab.
2. Adjust the x axis of the results graph from 10 to 100.
3. Obtain a control tracing.
4. Alter the maximum sodium conductance by increasing the values from 120 mS to 200 mS in 20 mS increments.
5. Record your results:

Conductance:	120 mS	140 mS	160 mS	180 mS	200 mS
Voltage:					

6. Repeat steps 1 and 3
7. Decrease the maximum Na^+ conductance from 120 mS to 40 mS in 20 mS increments.
8. Record your results

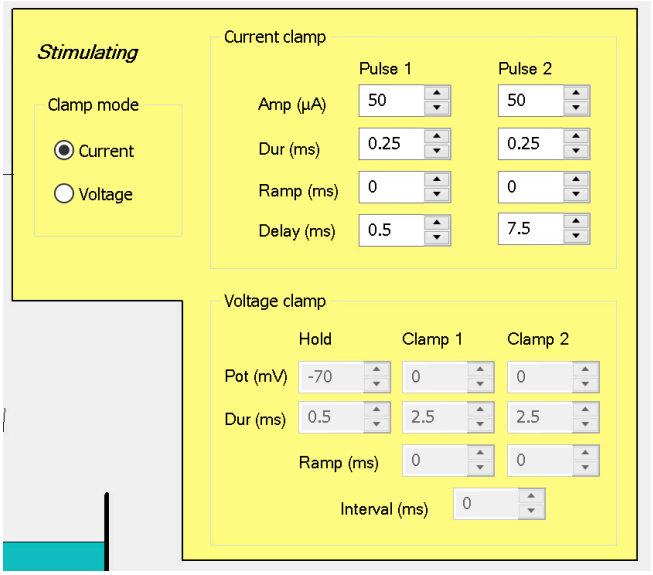
Conductance:	40 mS	60 mS	80 mS	100 mS	120 mS
Voltage:					

Questions (Exercise 6)

1. What happened to RMP? What do these two experiments tell you about the relative conductance of these ions at rest?
2. What is the physiological relevance of decreased K^+ conductance and increased Na^+ conductance?

The Action Potential

For your reference, here are the default settings for the next couple of experiments:



Exercise 7. Effect of stimulus strength

1. Return all values and settings to default conditions and clear your results.
2. Change the x axis to 25 msec.
3. Click on the "Traces" button within the results window, and make sure tracings for voltage, stimulus, and conductance are turned on.
4. Within the yellow current clamp window, change the amp for pulse 1 and 2 to be 0.
5. Generate a control tracing.
6. Increase the stimulus strength for pulse 1 in 5µA increments until you generate an action potential.

Questions (Exercise 7)

1. What is the underlying physiological basis for threshold.
2. At what point was an action potential generated (threshold)?

Exercise 8. Effect of stimulus duration:

1. Clear your results from last experiment.
2. Return to the setup window and set the stimulus strength at 35µA for pulse 1, while leaving pulse 2 at 0 µA
3. Obtain a control tracing.
3. Decrease the duration of the stimulus (default is 0.25 msec) in 0.05 msec increments. (You actually need to type this in the box as the arrows decrease the duration in .5 msec increments)
4. Record the minimum duration necessary to elicit an action potential. Record the strength and duration.
5. Continue until you reach a duration at which it is not possible to generate an action potential, regardless of the stimulus strength.
5. Clear your results.
6. Decrease the duration by another 0.05 msec and then gradually increase the stimulus strength until an action potential is again generated.
7. Record the strength and duration for this point.
8. Reset strength and duration to the conditions recorded in step 4 above.
9. Decrease the strength of the stimulus by 5 µA and gradually increase the duration until an action potential is generated. Record the data.
10. Continue decreasing strength in 5 µA increments until it is not possible to elicit an action potential regardless of the duration of the stimulus.
11. Plot your data points with duration on the y axis and stimulus strength on the x axis.

Questions (Exercise 8)

1. What is the relationship between stimulus strength and duration in the generation of action potentials? In other words, if we were to increase the duration of the stimulus, what would happen to the required strength of the stimulus in generation of an action potential?
2. How do stimulus strength and duration relate to how action potentials are generated in living systems?

Exercise 9. Effects of various drugs tetrodotoxin (TTX). TEA and Scorpion Toxin

1. Return all values to default settings (35uA and 0.25 msec).
2. Obtain a tracing of a single action potential.
3. Add TTX to the preparation and obtain a tracing.
4. Turn off TTX and add TEA to the preparation and obtain a tracing.
5. Turn off TEA and add Scorpion Toxin to the preparation and obtain a tracing.

Questions (Exercise 9)

1. What do each of these toxins do to the different ion channels involved in an action potential?

Explain how these toxins change the action potential (Feel free to utilize google here)

2. Based on the results, which ion (K^+ or Na^+) has the greater effect on membrane repolarization?

Exercise 10. Determination of refractory periods

Nerves and other excitable cells very seldom communicate using only a single action potential. Instead, "trains" of action potentials are used to carry specific messages. The duration of the action potential trains and the frequency of action potentials per unit of time, are important components of neural communication. Consider the pacing of the human heart during extreme activity. Each beat is generated by action potentials, and the rapid heart beat must necessitate a fairly high frequency of AP's to function. The flight muscles of an insect is an even more extreme example. Ultimately, there is an upper limit to action potential frequency. The goal of this exercise is to experimentally determine what factors control how close in time individual action potentials can occur with respect to each other. In other words, what is the maximum number of action potentials per unit of time?

1. Return all values to default settings and clear your results.
2. Set the x-axis to 50 msec
3. Set the interval (the delay between stimulations) to 30 msec by setting the value for the 2nd pulse, leave the first pulse at the default setting.
4. Set the amps on the first and second pulses to 35 mV.
5. Obtain a control tracing.
6. Gradually decrease the interval for pulse 2 in increments of 3-5 msec at a time.
    - At some point you will fail to obtain an action potential from the second pulse. This failure to obtain an action potential even though the voltage was sufficient previously means the nerve is refractory. There are two kinds of refractory states (or periods) for nerve cells: **relative refractory period** and **absolute refractory period**. If the nerve is in a relative refractory period you should be able to obtain a second action potential by either (1) increasing the duration of pulse 2, or (2) increasing the stimulus strength of pulse 2. When the increment in time is decreased to the point that neither duration nor strength of the second pulse is sufficient to generate an action potential, the membrane is in the absolute refractory period.

Question (Exercise 10)

1. Explain, physiologically (ie. membrane channels) the difference between relative and absolute refractory periods.
2. Based on the data you collected what would be the maximum possible frequency of action potentials in the squid giant axon?

Exercise 11. Effects of temperature on action potentials

Even mammals, which have the ability to achieve a relatively constant body temperature regardless of external temperature, experience some differences in body temperature. Nervous tissue, indeed, all

excitable tissue, is particularly sensitive to changes in temperature.

1. Return all values to default settings and clear your result.
2. Set the stimulus strength to 27uA and the temperature to 6 degrees
3. Predict, then record a tracing
4. Set the temperature to 3 degrees
5. Predict, then record a tracing
6. Set the temperature to 10 degrees
7. Predict, then record a tracing

Questions (Exercise 11)

1. What effect does temperature have on the action potential? Explain your results.