



Virtual Lab Student Experiment User's Manual

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Virtual Laboratory

Student Experiment Manual

A Collection Candidate Module by

Robert Macey Tim Zahnley

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This simulation program is based on the Nobel Prize winning Hodgkin-Huxley model for excitation of the squid axon which set a new paradigm in neurophysiology. The program simulates an excised axon from a squid and allows you to perform experiments by applying stimuli or clamps after setting the environment of the axon, changing its properties, and/or adding drugs or toxins. Using the program tools you can create experiments which explore a variety of nerve properties, ranging from classical phenomena such as threshold, summation, refractory period, impulse propagation to more modern concepts of channels, gates, and eventually even molecular events. This simulation provides insight about the hypothesized mechanisms of excitation in a way that is not practical with animal preparations. These ideas can be explored at both elementary and advanced levels. You can dive into a full blown propagating action potential with 15 recording electrodes or you can begin with an inexcitable axon and gradually patch in the component parts. In every case there are animations linked to the computations which will help you interpret any experiment that you specify. The experiments described in this manual are merely suggestions. You are encouraged to move beyond these limitations by proposing your own problems and experiments.

The program is feasible because computations are performed with an ultra high speed software engine specialized for very rapid computation of solutions to both ordinary and partial nonlinear differential equations. Linking this engine to a flexible multimedia object oriented development program has provided a programming environment that is both powerful and unique.

SUMMARY OF MODELS

Elementary and Advanced Models

These are exactly the same simulations; they differ only in their presentation. Elementary models are limited in the number of variables that can be plotted and in the number of parameters that can be changed.

Elementary models can plot 4 variables:

- a. Stimulus
- b. Membrane Potential
- c. Na and K Equilibrium Potentials

They can change:

- a. The stimulus parameters for two stimuli
- b. The concentrations of Na and K on either side of the membrane
- c. Temperature



These variables and parameters are intuitive and easily grasped by a beginner. Nevertheless, elementary models still provide a large array of experimentation.

In contrast, advanced models allow plotting of 13 different variables and changes in at least 22 parameters. The number of possible combinations is staggering.

Propagated A P / 1 E

This simulates the simplest experimental scenario. An axon is stimulated at one discrete site initiating a nerve impulse. The impulse travels from the site of stimulation and passes another site downstream where it is recorded by an intracellular electrode. This is the opening default model.

Using the elementary version a beginner can study:

- a. Threshold stimulus intensity
- b. All or None Response
- c. Strength Duration Curves
- d. Refractory Periods
- e. Temperature dependence of the size and shape of the action potential
- f. Dependence of any of the above on Na and K.

In particular he/she can study how the resting potential depends on K (but not Na) while the peak of the action potential depends on Na (but not K). This was one of the major clues prompting the formulation of the modern theory of excitation.

Using the advanced model allows a more detailed analysis of the above as well as the opportunity to do many more experiments. For example, an advanced user could also invoke changes in:

- a. Membrane capacitance
- b. Numbers of Na, K, or "Leak" channels
- c. Rate constants for either opening or closing of any or all of the 3 gates residing in the Na or K channels,

Propagated A P / 2E

This model is similar to the above, Propagated A P / 1E. But, now there are two recording electrodes at different positions along the axon. By dividing the time between peaks into the distance between the electrodes the user can calculate the velocity of propagation of the impulse independently of any uncertainties about the latency of excitation. The dependence of velocity of conduction on axon radius, temperature, Na and K, membrane capacitance, etc. can be studied.

Membrane Action Potential

Here, in contrast to the Propagated A P simulations, the entire axon is stimulated simultaneously. There is no propagation because there is no place for the



impulse to go. This is shown in the screen animation where large electrodes elicit an excitation uniformly over the entire stretch of axon. This experimental setup is very useful because it isolates the excitation process from propagation making it easier to interpret. (There are no up- or down- stream currents.) It can be used to study all of the properties discussed in the first model, but from a different perspective. Further the experimental setup is one step closer to a voltage clamp and makes the passage to voltage clamping seem more natural.

Passive Axon: No Voltage-Activated Gates

This model simulates the Membrane Action Potential experimental setup applied to a primitive inexcitable axon. The axon leaks Na, K, and possibly other ions through inert channels, but these channels have no voltage activated gates; the channels do not open or close in response to membrane voltage. The axon has a normal ion distribution (high K on the inside and high Na on the outside). It has a nearly normal resting potential because K ions leak out faster than Na ions leak in. This simulation shows

- a. The axon is inexcitable
- b. Its response is symmetrical
If you change the sign of the stimulus, the response also changes sign but has the same size and shape
- c. Its response is linear
If you sum stimuli you sum the response.
- d. Its response time depends on the membrane capacitance.

Passive Axon: Only K Gates

This model simulates an axon that has intact voltage activated potassium (K) channels, but no voltage activated sodium (Na) channels. This is one step toward the construction of a fully functioning axon. The axon is still inexcitable but its response is now nonlinear and non-symmetric. The slow accommodative effects of K channels are evident as the response rises to a maximum and falls away despite the continued presence of a prolonged stimulating current. The seeds of a refractory period are also evident from the undershoot that occurs when the stimulus is removed. This is an ideal time to introduce voltage clamping because interpretations involve only one significant ion with a simple channel with only one gate.

Passive Axon: Only Na Gates

This model, another step toward a fully functioning axon, is the complement of the last one (Passive Axon: Only K Gates). Now all the K channels are blocked and only Na channels remain. These channels are more complex because they contain 2 different gates, a fast one and a slow one. The absence of K gates makes interpretation of Na Channel behavior much simpler. This axon is excitable, but only if it has been artificially hyperpolarized just prior to the stimulus.



Further once it is stimulated it will remain depolarized with no prospect of re-excitation unless it is artificially hyperpolarized. Voltage clamping is, again the ideal way to study the opening and closing of the two channel gates.

After studying the last three models the user is prepared to tackle a more detailed description and interpretation of intact axon behavior. This can be accomplished by reopening Membrane Action Potential: Advanced and running the simulation with close attention to the gate animation.

Propagated A P / 15E

This simulation provides a 3d graphic visualization of the all-or-none wave like nature of impulse propagation, by recording from 15 sites along the axon

It also shows how subthreshold stimuli decay with distance from site of stimulation. (Best seen in 2d , but can also be done in 3d with zoom.) This type of experiment is useful in understanding propagation; it gives a measure of how far downstream a local disturbance can reach and is important for understanding impulse conduction. The space constant and its dependence on other parameters can be evaluated from this data.

Propagated A P / 1 5E/Smid

Again recording from 15 sites, the axon is stimulated in the middle rather than at one end. The simulation provides a nice visual demonstration in 3d that the action potential travels in both directions from the site of stimulation.

Propagated Action Potential/15E/ S2ends

Now the axon is stimulated at both ends so that the two impulses run into each other. They annihilate each other because they run into each other's refractory period. This shows the importance of the refractory period in excitable structures, particularly the heart where it prevents chaotic impulses from taking control.

Pharmacology: Na Channel Blocker

This simulation provides a demonstration of a local anesthetic acting on a segment of the axon. The simulation shows action potential records before, after, and at the site of the block. Movement of the slider shows different degrees of block. Conduction fails when the height of the record at the site of the block falls to about 1/6 of the control. This corresponds to the textbook figure of a safety factor of 6 for conduction of an impulse (i.e. each normal action potential generates 6 times the current required to excite the next region).

Toxicology: Scorpion Toxin

This simulation provides a demonstration of the effects of alpha scorpion toxin.



Voltage Clamp: Ideal

This model presents a standard voltage clamp simulation where voltage control is obtained by setting the membrane voltage equal to the target voltage. This is the companion to Membrane Action Potential

Voltage Clamp: Simulated

In this voltage clamp simulation the voltage control is simulated. This includes a user controlled gain together with an unavoidable delay. The system can show steady state error, oscillations and instability. It is a useful exercise for demonstrating common properties of feed back control.

Voltage Clamp: Only K Gates

This is the companion to Passive Axon: Only K Gates.

Voltage Clamp: Only Na Gates

This is the companion to Passive Axon: Only Na Gates

Voltage Clamp: Scorpion Toxin

This is the companion to Toxicology: Scorpion Toxin.

Problem 1...

These models present the user with two axons. One is a normal control axon, the other has been perturbed by changing one of its parameters. The user is asked to give an empirical description of the perturbation and then to perform tests to try to determine the nature and the extent of the perturbation.

OPEN PROPAGATED A P / 1E: ELEMENTARY

RUN THE SIMULATION

1. Click **Run button** on stimulator.

This button will always initiate a new run, independently of whatever running mode (multiple runs, sliders etc.) has preceded it, or whether the Slider palette is active. The screen animation shows an action potential initiated in a discrete position under the stimulating electrodes and traveling to the recording electrodes where it is picked up and displayed in miniature on the small recorder.

2. Click **Display button** on recorder for a larger view.

A Slider palette (window) will be floating on top of the graph.

3. Drag the **Sliders** window out of the way, e.g. place in lower right hand corner.

CONTROL THE STIMULUS

1. Click **Stim button** on stimulator.

A dialog appears allowing you to control the intensity, duration, and time of onset of two independent stimuli. The second stimulus is used to explore refractory periods etc.

2. Enter 3, 100, and 0.6 for time of onset, intensity, and duration respectively for stimulus 2. Then click **Done**.

Note that the new stimulus pattern is plotted in the stimulator display.

3. Click **Run button** on stimulator.

RETRIEVE NUMERICAL VALUES

1. Click on the button labeled with a "cross" (the second button on the left on the recorder display). A graph cursor in the form of a vertical dashed line will appear on the display, and numerical values corresponding to the position of the cursor appear on the upper part of the display.

2. Drag the cursor back and forth. Numerical values of any plotted variable can be obtained directly from the display at any position of the cursor.



CHANGE THE EXPERIMENT

1 .Click on **New Experiment** in the lower menu and select **Membrane Action Potential: Elementary** from the popup menu list.

2.Click **Run** button.

Now, in contrast to the last simulation the entire axon is stimulated simultaneously. There is no propagation because there is no place for the impulse to go. This is shown very nicely in the screen animation where large electrodes elicit an excitation uniformly over the entire stretch of axon. (Note that the animated impulse is first green (color coded to Na) and then red (color coded to K) reflecting the initial increase in Na permeability followed by an increase in K permeability.) This experimental setup is very useful because it isolates the excitation process from propagation making it easier to interpret. (There are no up- or down- stream currents.)

USING SLIDERS AND OVERLAY

1 .**Change parameters:** Move **Slider** for **Stim 1 intensity** back and forth.

Sliders provide a quick way to change a parameter and run the model directly from the graph window. A run will be initiated after each slider drag when the mouse is released. Each time a new run is completed the plot in the display window is erased and replaced by results of the new run.

2. **Fine control:** Click on the **Fine** check box next to **Stim 1 Intensity** in the **Slider** window.

This will place the last position of the **Slider** in the middle of the bar and increase the sensitivity of the slider by a factor of 10. Move the **Slider** back and forth and forth. Toggle off the **Fine** control by clicking again on the Fine check box.

3.**Compare runs:** toggle (click) on the **O button** (on recorder) for overlay.

4.Once more, move **Slider** for **Stim 1 intensity** back and forth.

Now the display window is no longer erased after each run. Instead, each new run is superimposed on the results of previous runs. As long as "overlay" is toggled on, screen erasure will not occur. This holds independently of how you initiate the run e.g. with sliders or with the run button.

5.Get rid of Slider by clicking close box in upper left hand corner.

6.Retrieve Slider window by selecting it from Set Parameters on bottom menu.



SELECT PLOTTING VARIABLES

1 .Click on the **Stim** button in the graph window. The stimulus rectangular wave disappears.

2.Click on the **Stim** button again.

The stimulus rectangular wave reappears. Clicking any of these variable buttons on the graph toggles that variable on or off.

3 .Click on **Glossary** on the bottom menu bar and select **Models** from popup list.

This glossary defines all the variables and parameters that are used. Click **Done**.

ADJUST PLOTTING AXES AND SIMULATION TIME

1 .**Change the Y axis:** Click on the Stim button while holding the shift key.

The stimulus rectangular wave is now plotted on the left Y axis. Do it again; it shifts to the right axis. Holding the shift key while clicking a button moves the variable from one Y axis to the other

2.**Change the time (x) axis:** Click the Time button on the recorder.

A dialog appears which allows you to change the simulated time of the next run. A corresponding change in the time axis will appear in the next graph. The same dialog can be reached via the "Set Parameters" (select Control) menu in the bottom menu bar.

SCALING

1. **Auto Scale:** toggle E_{Na} and **EK** off the graph.

The scaling on the left axis is adjusted so that the other plotted variables are shown in greatest detail commensurate with their size. In this mode, each time a change is made the plotted variables will continue to occupy the full graph, but the numbers on the left axis may change.

2. **Fixed Scale:** toggle E_{Na} and **EK** on the graph.

E_{Na} and **EK** are both plotted as defaults. They are upper and lower limits to the action potential; they fix the scaling, making it easier to assess changes visually. In this mode, each time a change is made the numbers on the left axis remain fixed while the size of plotted variables may change. The next version will include an option for user defined scales.



3. Zoom: Click and drag the mouse to draw a rectangle over any portion of the graph you wish to view in detail.

When you release the mouse, auto-scaling is turned off, and the axis limits are adjusted so that the portion that was in the rectangle now fills the window.

4. Unzoom: Click the un-zoom (**Z**) button on recorder:

This button simply turns on auto-scaling for all axes.

ENLARGE THE DISPLAY

1 .Click the (up) **Arrow** button on the recorder:

The display is enlarged and the arrow button now points downward:

2.Undo the display enlargement: Click the (down) **Arrow** button on the recorder.

The display is returned to its original size. Arrow button now points downward

TOGGLE BUTTONS ON RECORDER

1 .Click on **Glossary** on the bottom menu and select **Buttons** from popup list:

This glossary defines all the buttons that are used. Click **Done**.

2.Try some of the toggle buttons on the recorder. In particular:

L toggles legend

P toggles parameter list

O toggles overlay

G toggles Grid

Up **Arrow**/Down **Arrow** toggles recorder size

AUTO COMPARE RUNS WITH DIFFERENT PARAMETERS

1 .Click on **Multiple** on the stimulator.

A Multiple Runs dialog box appears. Set it up as follows:

2.Select **Intensity1** (this corresponds to **Stim 1 intensity**) from the Parameter popup menu.

3.Specify 10 runs to be performed.



4. Enter 0 in the **Initial value** and 100 in the **Final value** fields, respectively.
5. Select the **arithmetic series radio button**.
6. Click **OK** to start the runs.

The program will automatically run the program 10 times and changing the intensity with each run as indicated in the parameter list.

This same routine can be used to compare variations of any parameter in the popup menu list.

Note that the resulting run(s) will be added to the graph if Overlay is on. If you want any previous runs to be discarded, be sure to turn off Overlay before starting the runs.

SET PARAMETER VALUES

1. Click on the **Na** or **K** near the axon.

An **Environment** dialog appears which allows you to change the concentrations of Na or K (inside or outside the axon) as well as the temperature.

2. Click on the **Set Parameters** menu and select "whatever" from the popup list. All parameters that are user accessible can be changed in the dialogs from the popup list.

ADVANCED MODELS

1. Click on **New Experiment** and select **Membrane Action Potential: Advanced** — run it and click on Display on the recorder

More plotting variables are accessible in the advanced models than in the elementary ones.

2. Click on **Glossary** on the bottom menu and select **Models** from popup list

This glossary defines all the variables and parameters that are used. Click **Done**

3. Click on the **Set Parameters** menu and select "whatever" from the popup list.

More parameters and more dialogs are accessible in the advanced models.



MULTI-ELECTRODE MODELS

1. Click on **New Experiment** and select **Propagated A P /15E: Advanced**
Run it and click on **Display**.

This shows the propagated action potential as recorded at 15 equidistant sites along the axon by 15 independent electrodes.

2. Click on **3D** button on recorder.

The graph is now redrawn in 3d (pre-renaissance) perspective. The Y axis now shows distance (cm). (However there is a voltage calibration on the right of the graph). At the left there is a miniature axon running vertically. Recording electrodes icons (R) are placed in register with the plot that shows the recording at that position. The S icon shows the site of stimulation. By dividing the time between peaks into the distance between their respective electrodes you can calculate the velocity of the nerve impulse. Its easier to do (more resolution) with **Propagated A P /2E**try it!

GET HELP

1. **Button** and **Model Glossaries** are described above

2. Click on **Interpret** from bottom menu and select Explain from the popup.

Follow the explanation of the **Gate Animation**. The same interactions for any experiment can be accomplished in the following step.

3. RUN any model with any parameters that yield a full action potential.

4. Click on Interpret from bottom menu and select Gate Animation from the popup.

Drag the graph cursor (dotted vertical line on the left in the graph) and note how both the driving force arrows and the channel states change. These forces and states correspond to the position of the cursor and are used to interpret each detail of the graph. This animation is quantitative; it will interpret the details and unique properties of any simulation

5. Click on **Interpret** from bottom menu and select **Equilibrium Potential** from the popup.

6. Click on **Interpret** from bottom menu and select **Explain Voltage Clamp** from the popup.



Experiment 1: Properties of Excitation and Conduction

Overview

A. Threshold and All-or-None Response

If a nerve is stimulated with weak electrical shocks there is a local disturbance but a propagated action potential does not occur. As the stimulus intensity is raised the local disturbance gets larger and finally, at a critical intensity or threshold, an action potential occurs and is transmitted along the length of the nerve. In contrast to local disturbances, it is much larger and its height does not diminish as it travels along the length of the axon. Further increasing the stimulus strength does not increase the size of the propagated action potential. This behavior is called all-or-none. In this experiment you will find the threshold of a nerve and see what the action potential looks like. Then you will raise the stimulus strength to values above threshold and observe the effect upon the magnitude and shape of the action potential.

Task

Open the Virtual Lab by double-clicking on its icon. Click on Propagated Action Potential when it appears on the screen. When the experiment is loaded you will see a screen with an axon, recording instruments and a slider for changing parameters (see fig. 1).

As you can see from the slider, the initial stimulus parameters are $200 \mu A/cm^2$ for the intensity and 0.25 msec for duration. (These are the values for the first stimulus. Ignore the second stimulus; its strength has been set to 0 so that it will not occur in this experiment. The stimulus pattern is displayed on the small stimulator screen on the left. To stimulate the axon click the Run button on the stimulator. In a few moments, after you have stimulated the axon on the left and after the impulse passes along the axon to the recording electrodes on the right, you should see an action potential plotted on the small recorder on the right.

Click the Display button on the recorder. This will enlarge it and make it easier to view the action potential trace. (To further enlarge the view click on the upward pointing arrow at the bottom of the screen. Clicking on a downward pointing arrow will reduce the view). Drag the slider to the bottom of the screen where it will not obscure the view. You are now ready to do the experiment.

Before going on print a copy of the screen and take a look at your action potential . To do this:

- if you want a grid click the G toggle button
- click the Print button
- set the Reduce or Enlarge option to 25%
- print

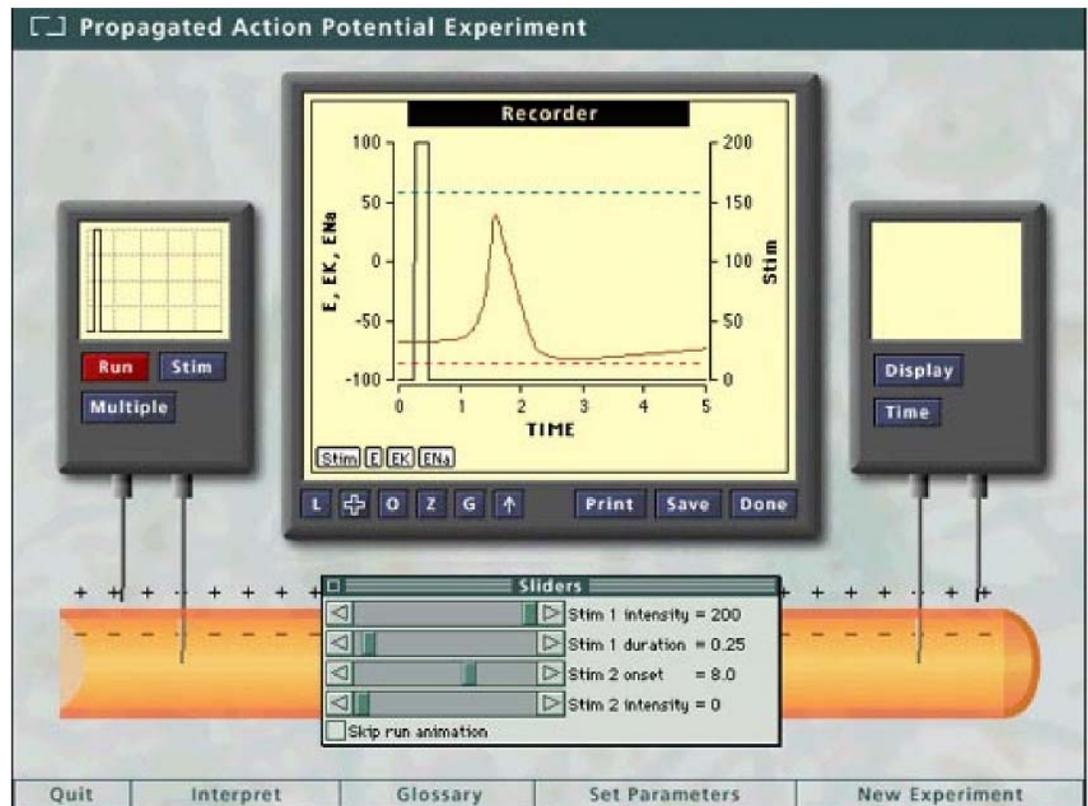


Fig. 1.1 Virtual Lab Screen, showing the recorder, slider and control buttons.

Make notes of what you see on the printout. Notice the initial negative resting potential. Observe that the action potential spike shoots past 0 and becomes positive. As the nerve recovers notice the negative afterpotential. It is more negative than the resting potential. Notice that the entire action potential is bounded by 2 lines labeled EK and E_{Na} . These symbols stand for the equilibrium potentials for K^+ and Na^+ ions, respectively. It was known, even before the mechanism of the action potential was understood, that these 2 ions were essential for nerve activity. We will come back to this point in later experiments. Take readings of the voltages at the resting potential, peak of the action potential, and lowest point of the afterpotential. There is an easy way to do this:

- bring the mouse cursor onto the screen- it will turn into a cross
- bring the cross slightly above the point of interest, say the action potential peak
- click and hold the mouse switch down; then drag a box around the point
- when you release the mouse switch the recorder will zoom in to the region within the box



- click the  toggle switch; this will put a moveable graph-cursor (a vertical dashed line) on the screen
- move the mouse with the button held down to center the graph-cursor on the point of interest. The time in msec and the voltage in mV will appear at the top of the screen.
- to get out of the zoom mode click the Z toggle switch.

Record the values for your action potential:

resting potential = _____ mV

action potential peak = _____ mV,
 occurring at _____
 m s e c

afterpotential minimum = _____ mV,
 _____ occurring at _____
 _____ m s e c

Note that the stimulus starts at 0.25 msec in these experiments.

Since you produced an action potential, the initial stimulus intensity of 200 was obviously above threshold. Now lower the magnitude of the stimulus to about 100 by dragging the slider to the left and release the mouse button. Each time you release the mouse after moving the slider the stimulator will automatically activate as though you had clicked the Run button. In this case a new action potential results; 100 is still above threshold. Now, again move the slider to the left, and lower the stimulus to a very low value , say 10. No action potential will occur because the stimulus is subthreshold. Test different values of stimulus strength by moving the slider until you find the lowest value that will elicit an action potential. Now click on the check box labeled Fine in the slider window. This will make the slider more sensitive and enable you to find a more accurate lower bound for an adequate stimulus. To the Record the value that you find. This is the threshold strength for a duration of 0.25 msec. (Do not change the stimulus duration in this experiment. We will come back to duration later). Why do you think there is a threshold in nerve stimulation? Are there any other phenomena in nature like this? You might consider a chemical reaction or the lighting of a fuse.

Compare the spike height of an action potential elicited with a threshold stimulus with those of action potentials elicited by greater stimuli. Are the heights the same? Do the action potentials differ in shape? A good way to compare the action potentials is to click on the O (Overlay) button at the bottom of the recorder screen. This will superimpose records from different experiments. (It is easier to compare the records if you reduce the display time to 5 msec. To do this click the Time button on the recorder. This opens a dialogue box that allows

you to reset the display time). When you have a graph that answers the questions print it and make notes on it as before.



Overview

Since this is the first experiment it is a good time to experiment with the various controls to see what they do. You have used many of them already. Consult the Virtual Lab Quick-Start for guidance on the others and try all of the controls. Note that the first set of buttons below the screen (Stim, E, ENa, EK) are toggles that allow you to change what is plotted. The lower set (L, + O, Z, G, ↑) allow you to add legends, control the graph-cursor, overlay, unzoom, add a grid or change display size. Other buttons in this row allow you to Print or Save graphs. If you click Done the graph will be reduced to its original small size. At the bottom of the screen are pop-up menus that allow you to Quit, Set Parameters or go to a New Experiment.. If you click the Interpret or Glossary buttons you will get information about the definition of symbols and the interpretation of the results you see on your screen. Selecting the Multiple button on the stimulator will open a dialogue box that will let you program the computer to do several sequential runs in which one of the parameters is systematically varied (in this experiment we did this with the slider and the overlay feature). The Stim button allows you to change stimulation intensity and duration (we used the sliders in this experiment instead).

B. Strength-Duration Curve

Task

In the previous simulation we found that a threshold stimulus intensity was required to fire a nerve. In this simulation we did not vary the stimulus duration and this raises the question: can you compensate for a weak stimulus by applying it for a longer time? It is tempting to answer yes. Depolarization of a nerve requires movement of charges across the cell membrane and if you move the charges slowly (weak stimulus intensity) it should take a longer time to reach threshold (long stimulus duration). Try the simulation and see what happens.

Load the Propagated AP/1E: Elementary experiment as you did in the first experiment. Set up the Display and move the slider out of the way.

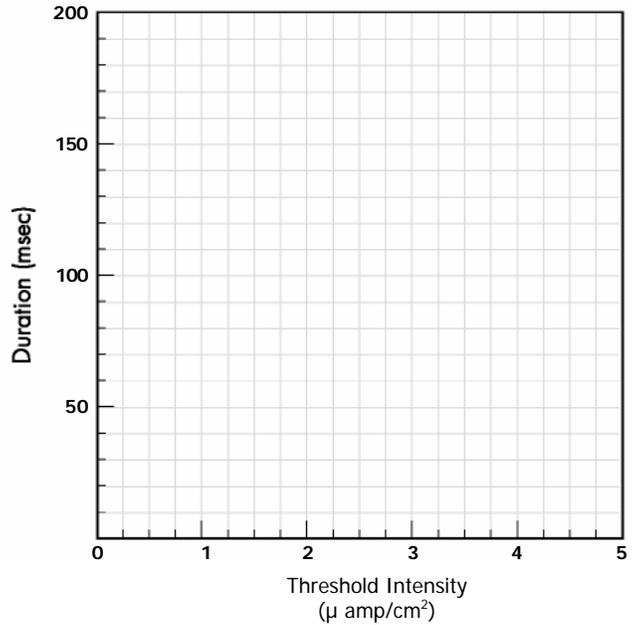
Set the stimulus duration to different values and determine the threshold intensity for each. The strength and duration values may be varied with the slider. To get values not accessible from the slider, click the Stim button. When the dialogue box appears type in the values that you want. Record your values in the table below, and plot the threshold strength vs the pulse (stimulus) duration in the graph to obtain a threshold intensity (strength) vs duration curve. This is often simply called a strength-duration curve.



Overview

Strength Duration

Duration	Threshold Intensity



Notice that no matter how long you stimulate, a minimum value of stimulus intensity is required. The minimum strength below which a nerve cannot be stimulated is called the rheobase. Mark it on your graph. Also mark the duration required if the nerve is stimulated at twice the rheobase value. This duration is called the chronaxie.

If you did the experiment correctly you saw that the weaker stimuli required longer durations to reach threshold. So, the qualitative answer to the question raised in the introduction is yes- if the stimulus is weak you can compensate by using a longer duration. However, this is only true within a certain range. Below the rheobase increasing the stimulus duration has no effect.

C. Refractory Period

After a nerve has been fired there is a short period of time during which it is inexcitable. This is called the absolute refractory period. Following this there is a period of time during which the nerve can be fired, but only with a stronger stimulus. This period is designated the relative refractory period. Refractory periods are determined by giving a second stimulus just after the appearance of the action potential. In this simulation we will determine the refractory period of the nerve we studied in the previous 2 simulations.



Task

Again, load Propagated AP/1E: Elementary. Set up the display as before. We will use 2 different procedures to check the refractory period. The first will use the multiple run capability of the program. Click on the Stim button and when the dialogue box opens set the durations of both stimuli to 0.25 msec. Set both intensities to the threshold value for this duration (about 70). Next click on the Multiple button . The following dialog will appear. At Number of runs type 7.

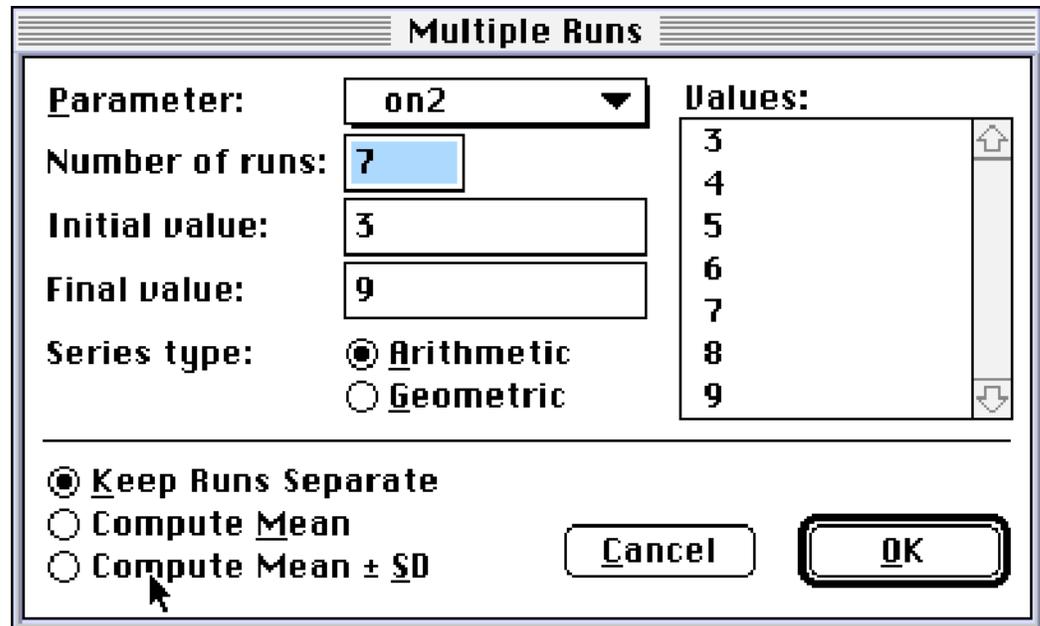


Fig.1.2 Multiple Run dialog box.

Select on2 (an abbreviation for Stim 2 onset) from the Parameter popup menu. This specifies that we will set a sequence of times when the second stimulus is to be applied. Set the initial run at 3 msec and the final at 9 msec. Choose the Arithmetic Series Type . This will give 7 runs with the second stimulus delivered at 3, 4, 5, 6, 7, 8, and 9 msec after the run starts. This is indicated in the Values list in the dialog on the right. The runs will be superimposed upon a single graph. Click on OK and watch the curves as they are plotted. For the first few the second stimulus will not elicit an action potential. As the period between stimuli increases a second action potential will appear. Since this is a threshold stimulus, when a second action potential occurs you know the stimulus was beyond the refractory period. Print out your graph and on it mark a rough estimate of the refractory period. To get it closer you will have to stimulate at shorter intervals.

Raise the stimulus strength to the highest value available, 200, and repeat the experiment. Did action potentials occur at intervals where they did not in the first set of runs? If a large stimulus elicits an action potential at a time where a

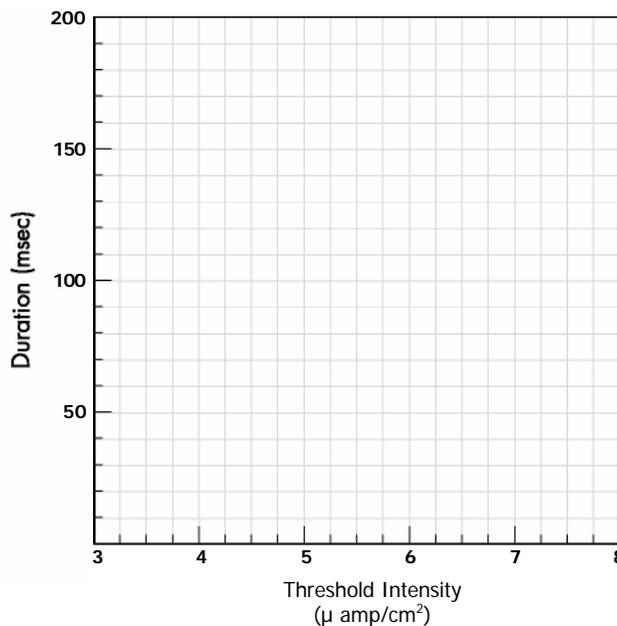


Experiment 1: Properties of Excitation and Conduction

threshold stimulus does not, is the axon in the absolute or relative refractory period? Print your graph and mark the range which is in the relative refractory period.

In the second procedure we will measure the thresholds at different time periods after the first action potential. To do this leave both stimulus durations at 0.25 and set the first stimulus intensity at the threshold value (about 70). The second stimulus intensity will be varied using the slider. Set the Stimulation 2 Onset at 3 msec to start. Vary the stimulus strength with the slider to determine the threshold. Write your findings in the table below. (If you cannot elicit a second action potential with the highest intensity write "ref" in the table. Increase the onset time by 0.5 msec and determine the threshold at this time point. Repeat until you have threshold values from 3 to 8 msec. (Note: In the experiment the first action potential peak occurs at about 1.6 msec, so a second stimulus at 3 msec is given 1.4 msec after the peak). Plot the threshold intensity vs time. At what time is the refractory period over for this nerve? How does the refractory period of a nerve set the maximum rate at which a nerve can fire?

Duration	Threshold Intensity
3.0	
3.5	
4.0	
4.5	
5.0	
5.5	
6.0	
6.5	
7.0	
7.5	
8.0	



Thresholds at Different Time Periods Following Excitation

The existence of a refractory period is a special feature of an excitable membrane. A nerve membrane must recover from the previous impulse before it can fire again.



D. Temperature Dependence

Overview

All biological processes are sensitive to temperature and the nerve action potential is no exception. Raising the temperature increases the velocity of molecules and most often this speeds things up. In this experiment we will vary the temperature from 0 to 40° C and observe the effect on action potential parameters such as peak height and duration. Since the action potential is a complex process you should be prepared for some surprises.

Task

Load Propagated AP/1E: Elementary and set up the display. The easiest way to study temperature dependence of the action potential is to use the multiple run option. Click the Multiple button and choose Temperature as the parameter to be varied. To avoid cluttered graphs run one series from 0 to 20°C and a second series from 20 to 40°C. Use the Arithmetic Series Type and choose the proper number of runs to give curves 5° apart.

Print the two graphs and label each curve with its corresponding temperature. At what temperature did the squid axon stop functioning? Are you surprised? What is the approximate temperature range of a squid's natural environment?

E. Conduction Velocity

Overview

To determine the conduction velocity of a nerve it is necessary to measure the times at which an action potential arrives at 2 electrodes at different locations on the nerve. Mammalian nerves have conduction velocities in the range of 1 to 100 meters/sec. Compare these numbers with those you obtain from this large invertebrate nerve.

Task

Load the Propagated AP/2E: Elementary and set up the display. This simulation will allow you to view the action potential from 2 electrodes, one 0.8 cm and the other 2 cm from the point of stimulus. Notice that distance along the axon is now plotted on the Y axis. The axon is represented by a vertical orange stripe located just to the left of the graph. A small icon marked S is placed at the beginning of the axon (where distance = 0) to represent the point where the stimulus is applied. One icon marked R for "recording electrode" is placed at a distance of 0.8 cm from S, another is placed at 2 cm.

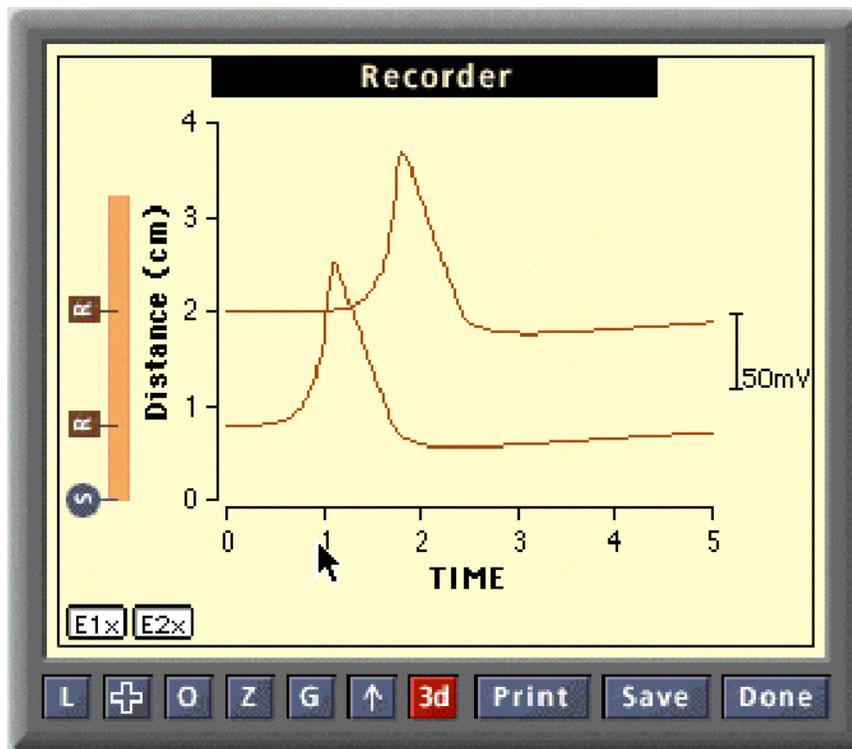


Fig. 1.3 Recorder display.

To measure the conduction velocity in meters per sec. you need the time at which the peak reaches the 0.8 cm electrode and the time at which it reaches the 2.0 cm electrode. This distance = 2.0 - 0.8 = 1.2 cm = .012 meters. The relevant times in msec are easy to read off of the graphs using the zoom and graph cursor features. To convert to seconds, multiply the msec by 0.001. Measure the times for temperatures from 0 to 30°C at 5° intervals. To change temperature in this simulation use the Set Parameters button. Choose Environmental and this will provide a menu to change the temperature.

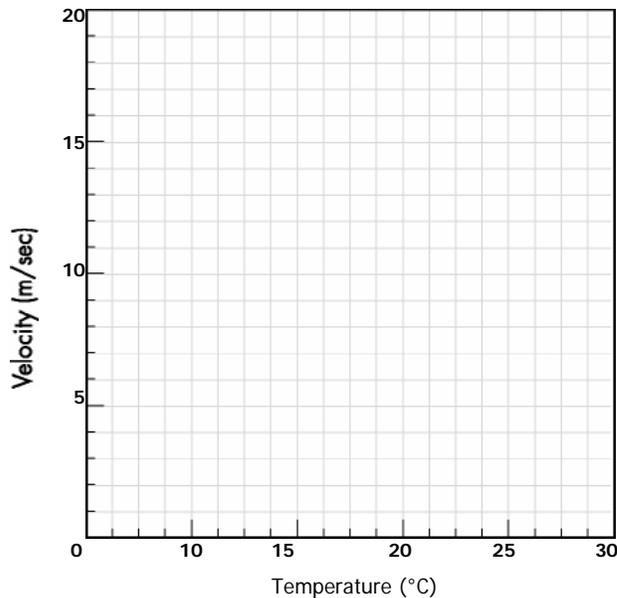
Calculate the conduction velocities from the equation:

$$\text{Conduction Velocity (meters / sec)} = \frac{\text{distance (meters)}}{\text{time (sec)}} = \frac{0.012(\text{meters})}{\text{sec}2.0 \pm \text{sec}0.8}$$



Record the data in the table below and plot the graph:

Temperature	Time at 0.8 cm msec	Time at 2.0 cm msec	Velocity meters/sec
0			
5			
10			
15			
20			
25			
30			



Load Propagated AP/15E: Advanced and run action potentials at 25 and 30 °C. Print the graphs. What signs did you see of nerve failure at high temperature?

F. Conduction Velocity Depends On Radius

Action potentials are conducted along axons because each excited segment electrically excites the next adjacent region which, in turn, excites the following region. The speed of conduction depends on how far downstream the electrical effects of an excited region can reach. Given these facts, you might anticipate that axons with larger diameters will offer less resistance to the downstream propagation of these electrical effects so that larger diameter axons would conduct faster. In this experiment you will test this conjecture.

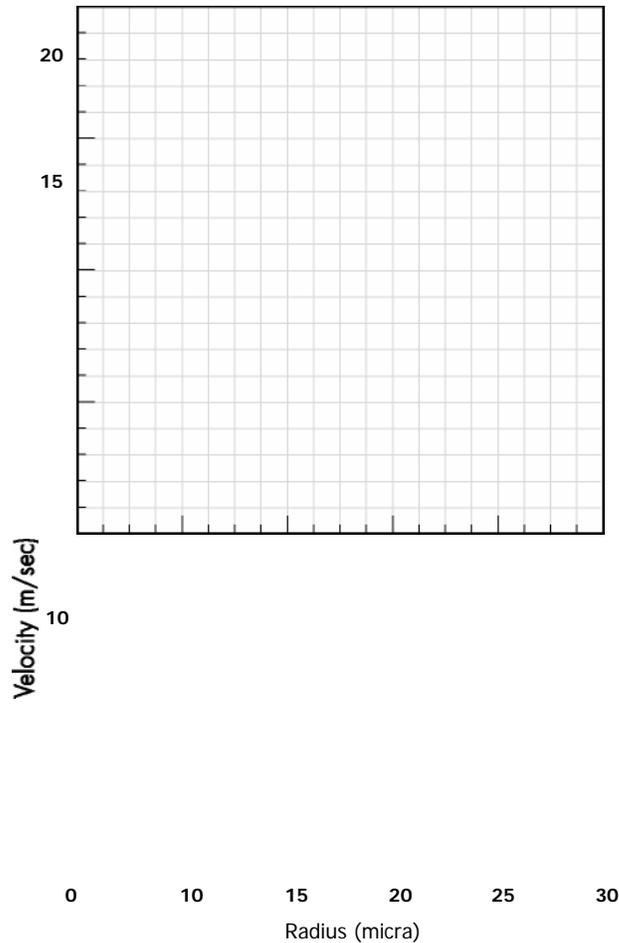
Overview



Task

Load the Propagated AP/2E: Advanced and set up the display. Measure the conduction velocity at different axon radii by filling in the table below and plot the graph. You can change the radii by using the Set Parameters menu and selecting membrane from the popup menu. Axon radius will be the last entry in the dialog box that follows. Alternatively you can choose Multiple runs, but to avoid screen clutter, do not try more than two runs at a time.

Temperature	Tlme at 0.8 cm msec	Tlme at 2.0 cm msec	Velocity meters/sec	Velocity meters/sec
0.005	50			
0.01	100			
0.02	200			
0.03	300			
0.04	400			
0.05	500			



A high conduction velocity is important for quick response; it has a high sur-

Experiment 1: Properties of Excitation and Conduction

vival value. How does the squid nerve compare with mammalian nerves?



Overview

G. Multi-Electrode Recordings

Wavelike properties of nerve impulse (action potential) conduction can be seen when we record from several sites. In these experiments we begin by stimulating at one end and observing the action potential as it passes different positions along the length of the axon. Next, we stimulate in the middle of the axon to check for one- or two-way conduction. Finally we ask what happens when two impulses arrive at a head-on collision

Task

Action Potentials Propagate As Waves

Load Propagated AP/15E: Advanced , set up the display, and run the simulation. Click on Display and then click on the up arrow to obtain the largest display. Looking at the vertical orange axon in the Display you will again see the stimulus icon S located at the beginning of the axon (where Distance = 0), but now there are 15 recording sites spaced 0.4 cm apart. The recording corresponding to each site is shown on the plot just adjacent to the site. The wavelike character of the propagated action potential is apparent on the plot, and it is easy to watch how it changes when any parameter is changed

Changes in temperature provide a good example. To prepare, first click on the Time button on the small Display (or alternatively select Control from the Parameter popup menu). Change the time setting in the dialog from 5 to 10 msec. Run the simulation and then click on the O (overlay) button on the Display . Now, change the temperature from 18.5° to 0° (select Environment from the Parameter popup menu to arrive at the dialog that allows temperature to be changed) Run the simulation again to obtain a graphic display of how temperature changes the shape of the action potential as well as its velocity of propagation.

One or Two Way Conduction ?

Load Propagated AP/15E: Advanced , set up the display, and run the simulation. Here the Stimulus is delivered to the middle of the axon (Distance = 3.2). Is the propagation confined to one direction, or does it travel in either direction with equal ease?

Colliding Action Potentials

Load Propagated AP/15E/S2ends: Advanced and set up the display. This program stimulates a nerve from both ends at the same time. The excitation advances towards the middle of the axon from each end and eventually the 2 action potentials will collide. If the action potential waves cross they will form an X pattern on the screen. This is what happens with ocean waves; they pass right through each other. Press the Run button and see what happens with action potentials. . Were the waves able to cross or were they blocked? Can you use the refractory period to explain this blockage? Why might the blockage of colliding action potentials be a useful feature for the heart?



Overview

H. Subthreshold Local Response

The nerve does respond to subthreshold stimulation, but the response is weak and diminishes from the point of stimulation. Subthreshold responses are important in physiology because they can summate with others, sometimes reaching the threshold magnitude. On the molecular level they produce disturbances in membrane channels that can affect the activity of the nerve. In this experiment we will look at a subthreshold response at 15 different electrodes as it spreads along a nerve axon.

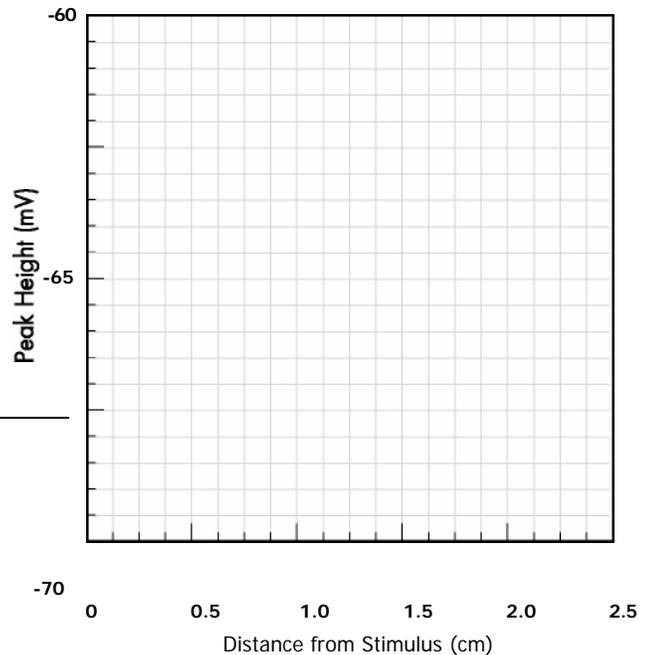
Task

Load Propagated AP/15E: Advanced and set up the display. Click the Stim button and reduce the stimulus intensity to the subthreshold value of 40. Click the Run button and observe the resulting 3D plot. What you see are 15 curves, each 0.4 cm from its neighbor. This plot does not show much because the scaling is poor. To get better scaling click the 3D Toggle. This puts the display into a 2D mode, superimposing the 15 curves. Next click the EK and E_{Na} Toggles to turn them off. You should see a nice display of the local response at different points as you move away from the stimulating electrode. Print a copy of the recording.

The highest peak occurs in the electrode 0.4 cm from the stimulus, the next highest 0.8 cm from the stimulus, and so on. Although you have 15 curves you will probably find only 6 or 7 of them of sufficient height to take measurements. Measure the peak heights of the useful curves (use the Zoom and Graph Cursor features),

plot the graph.

Distance from Stimulus (cm)	Peak Height (mV)
0.04	
0.08	
1.2	
1.6	
2.0	
2.4	



Notice that the subthreshold response curves do not decay directly back to their starting points but undershoot that level. This shows that the nerve is responding in an active fashion even in the subthreshold region.



Overview

I. Sodium and Potassium Effects

Excitation is based on the sequential movements of sodium and potassium ions across the membrane. But, how could you show this? How would you even show that they are important? One simple way is to replace them in the solution that bathes the nerve and see what happens to the action potential. A more subtle approach would be to replace them gradually (i.e. change their concentrations in steps). In this way the process (action potential) does not simply disappear. Instead it may become gradually distorted. If you can key the changes in concentration to corresponding changes in action potential, you may begin to acquire clues about what each ion does. In this exercise, you will simulate experiments originally done by Hodgkin, Katz & Huxley where they varied the external Na and K concentrations and observed the effect on the action potential.

Task

Load the Propagated AP/1E: Elementary experiment and set up the display.

Na Effects:

We will study the Na effects by varying external Na, which is the way the experiment was first done. Later people learned how to change the internal Na by perfusion, but this is technically more difficult and can be done only with the largest nerves. Click the Multiple button, set the number of runs at 6, and choose Nao as the parameter to be varied. You will find that the default value for all 4 of the ion parameters (Nao, Nai, Ko, Ki) is 1 .0. This simply means that the relevant concentration is 100% of normal. If you change it to 0,5 you will have 50% of normal while a change to 2 designates 200% of normal Set the initial value of Nao at 1 .2 and the final value at 0.2. This will give 6 runs at Naos of 1.2, 1 .0, 0.8, 0.6, 0.4 and 0.2.

Look at the 6 curves on your screen. There are 4 definite action potentials, a definite failure and one (the curve for Nao = 0.4) that could be either. How can you decide whether or not this curve is an action potential? Look at it for a minute or two to see if there are any distinguishing features. We will come back to this curve after we have analyzed the others. Print the graph and then use the Zoom and Graph Cursor features to measure the following parameters for each curve: peak height, maximum rate of rise, maximum rate of decline and negative afterpotential minimum. Put your data in the table:

NAo (x normal)	Peak Height (mV)	Max Rise Rate (mV/msec)	Max Decline Rate (mV/msec)	Afterpotential Minimum (mV)
1.2				
1.0				
0.8				
0.6				
0.4				
0.2				



Experiment 1: Properties of Excitation and Conduction

Which parameters are affected by Na the most? How much does the peak height change if you reduce external Na by half?

Now let's return to the 0.4 curve. It is impossible to tell whether this is an action potential or a local response from shape or size alone. A definitive test is to see if it is propagated in an all-or-none fashion. Load Propagated AP/2E: Elementary, set N_{ao} to 0.4 and find out. Is the electrical disturbance of the 0.4 curve an action potential?

K Effects: We now do a similar experiment in which K_o is varied, measuring effects on the same parameters as before. Start by resetting N_{ao} to 1.0. Vary K_o from 0.2 to 2.0. The design of the experiment is left up to you. Use single or multiple runs as you wish. The choice of the K_o values, other than the 2 extremes, is also left up to you. Choose values that will show significant effects. When you get your curves measure the same parameters as you did in the Na experiments. Put the data for 5 or 6 good curves in the table below:

K_o (x normal)	Peak Height (mV)	Max Rise Rate (mV/msec)	Max Decline Rate (mV/msec)	Afterpotential Minimum (mV)
2.0				
0.2				

Which parameters were most affected by K_o ? On one of your curves mark the Na and K sensitive parts of the action potential. Predict what will happen if you change internal K. Run a few curves to test your prediction.

Summarize all the evidence you have concerning the role of Na in the action potential.

Similarly, summarize the role of K in the action potential.

Data similar to that in your tables seems to point to the existence of special channels for Na and K that open and close during different phases of the action potential. We will examine the properties of these channels in future experiments.



Overview

J: Na Channel Blockers

Many local anesthetics act by binding reversibly to the membrane protein that forms the Na+ channel and blocking Na+ from entering the axon. The axon becomes inexcitable and cannot propagate an action potential. Procaine and lidocaine are examples. Other Na+ channel blockers, secreted by marine organisms, are classified as paralytic poisons, but their basic action is similar to local anesthetics. Tetrodotoxin derived from the puffer fish, and saxitoxin, from microscopic dinoflagellates are prime examples of these neurotoxins. The source of saxitoxin is particularly interesting, because these microorganisms proliferate in certain seasons to such an extent that they impart their reddish color to the water giving rise to the "red tide" At that time, shell fish feeding on them become contaminated with the poison and are not edible.

In this experiment we will apply a Na channel toxin at a single location in the middle of an axon and find out how much inhibition is required to block passage of the impulse.

Task

Load experiment Pharmacology: Na Channel Blocker and set up the display.

Find the slider which allows you to set the percentage of Na channels that are blocked. Set to percentage to 0. This will run the experiment and you will see 3 curves. Toggle the 3D button and you will see the nature of the experiment. The 3 curves are taken 0.8, 2.0 and 3.2 cm downstream from the stimulus. All 3 should be full scale action potentials at this point. In future runs you will block channels at the 2.0 cm electrode and see if the impulse can still get through to the third electrode.

Block increasing percentages of the channels at 2.0 cm and find the point where the electrical disturbance is insufficient to pass the action potential on to the 3.2 cm electrode. Record your observations in the table below:

% Na Channels Blocked	Peak	Height	at	2nd Acti

on Potential at
Electrode (mV)

3rd Electrode (es or No)



Think about your results in terms of the nerve's safety factor- the nerve can lose a lot of channels in a small patch and the impulse can still get through. Although the electrical response at the second electrode is diminished it still may be sufficient to excite areas around it which have not been anesthetized. How much can you diminish the height of the action potential before it will fail to excite the adjacent region? This is sometimes referred to as a "safety factor" for conduction.

Now look at your results from another perspective. Suppose that the nerve under consideration is a pain fiber and that you are trying to relieve a patient's pain by inhibiting it. When you have succeeded in blocking 50% of the channels you might assume that you had reduced the pain 50%. Would this be correct?



Experiment 2: Reconstructing the Axon: The Passive Axon

Overview

In the first set of experiments we investigated the electrical properties of nerves—the threshold, refractory period, temperature sensitivity, conduction velocity and so on. We found that these properties were very dependent on the concentrations of Na^+ and K^+ ions on the alternate sides of the cell membrane. To explain these ionic dependencies, it was suggested that ions carried charges across the membrane through special channels that could be opened and closed.

In the next set of experiments we will reconstruct the properties of a nerve. We begin by stimulating a primitive inexcitable axon. It leaks Na^+ , K^+ , and possibly other ions through inert channels, but these channels have no voltage activated gates; the channels do not open or close in response to membrane voltages. The axon has a normal ion distribution. The simulation shows the basic properties of a leaky membrane. In experiments that follow, voltage-dependent channels will be added for K^+ and for Na^+ . We will see how properties of the membrane change as these channels are added.

Task Testing the Inexcitable Axon

Load the experiment Passive Axon: No Voltage-Activated Gates. Notice that the interface has been modified; the stimulating electrodes run the length of the axon. In addition to simplifying the axon by removing the voltage activation of the channels we now employ a simpler experimental set up. Instead of stimulating the axon at one discrete location and recording the response at another site, we now stimulate the entire axon simultaneously. Run the simulation and watch the screen animation where large electrodes elicit an excitation uniformly over the entire stretch of axon. This experimental setup is very useful because it isolates the excitation process from propagation making it easier to interpret. (There is no propagation because there is no place for the impulse to go.)

This axon has an ionic distribution similar to a typical axon (high K^+ inside, high Na^+ outside). It has a negative membrane potential because the inert channels transport K^+ ions in preference to Na^+ and other ions. Record the membrane potential, E :

Start by varying the strength of the stimulus to see if you can produce an action potential. The initial values have been set at 50 mA/cm² and 2.5 msec. Try stimuli from about 20 to 200 mA/cm². For each stimulus record the maximum voltage in the table below. Reverse the signs of the stimuli and observe the results. Were you able to elicit an action potential? _____ . Now subtract membrane potential from each of the peak values in the table. Is the magnitude of the response symmetrical when you reverse the sign of the stimulus? _____

Are the shapes & magnitudes of the response curves symmetrical when you change the sign? Print graphs which support your answer.



When you are done load Membrane Action Potential: Elementary. Is the response symmetrical when you change the sign of the stimulus?

Symmetry

Intensity	Peak Voltage (mV)	Peak Voltage - E (mV)

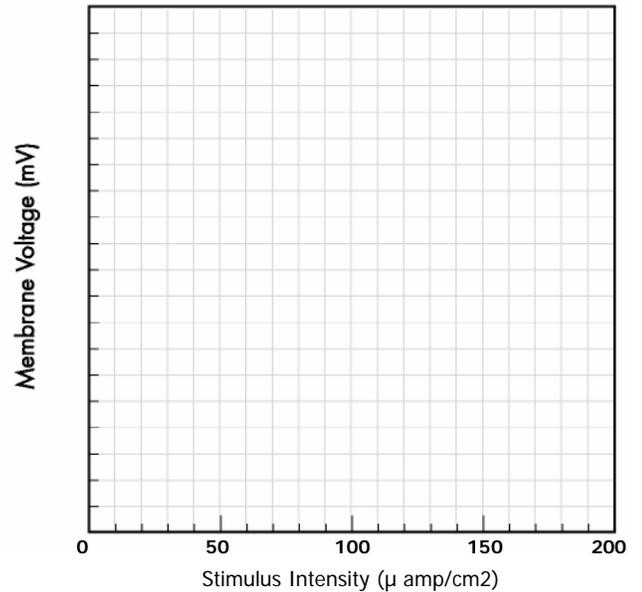
Testing for Linearity of Response

Reload Passive Axon: No Voltage-Activated Gates. Now change the duration of the stimulus to a longer value so that the response curves flatten out and reach a steady state. Duration used = _____. Keep this new duration constant, and vary the stimulus strength from 20 to 200 mA/cm². Record the values in the table below and then plot the membrane voltage vs stimulus strength to see if the response is linear (i.e., do the points fall on a straight line?).



Linearity

Intensity (μ amp/cm ²)	Peak Voltage (mV)



How does this response differ from the all-or-none response of a real axon?

Does the passive membrane have a threshold?

Testing for a Refractory Period

Use 2 identical stimuli to test for a refractory period in the passive axon. Use the slider to vary the Stim 2 Onset. Does the magnitude of the response change if you change the time interval between 2 pulses? _____. Is there a refractory period? _____

Response to Second Stimulus

	Stim 2 Onset	

(msec)
Peak Voltage (mV)

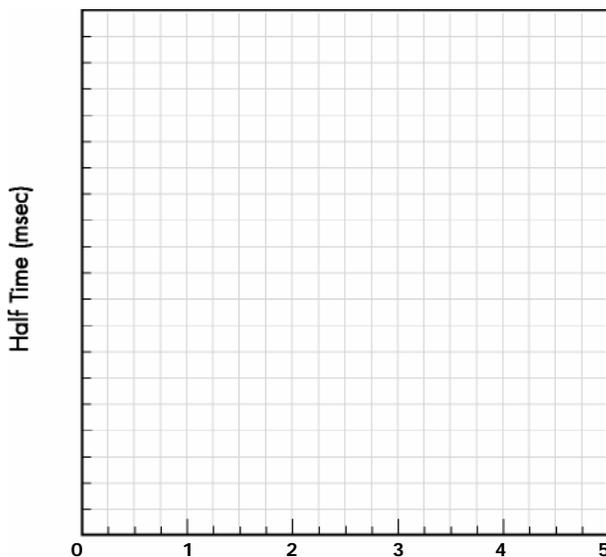


Testing the Effect of Membrane Capacitance on Response Time

Pick a stimulus strength and duration that will give a substantial change in membrane potential. The duration must be long enough for the response to reach a steady state. Run the simulation and record the resting potential and the value of the membrane potential response. Subtract the resting potential, E_m , from the membrane voltage response to get the magnitude of the voltage change. Next divide the voltage change by 2 to get the 50% response. Find the 50% response voltage on your graph and use the zoom feature to get the time of the 50% response. Subtract 0.5 msec from this time value (because the stimulus onset was at 0.5 msec) to get the half-time of the response. Record this value in the table. Now vary the membrane capacitance to see how it affects the half time of the response. To change the capacitance go to the Set Parameters Menu and choose the Membrane option. You may change the capacitance from 0.1 to 5 X the normal value. Plot the half time of response vs the membrane capacitance.

Response Time - Capacitance

Capacitance (x normal)	Membrane Voltage (mV)	Voltage Change (mV)	50% Response (mV)	Half Time (msec)





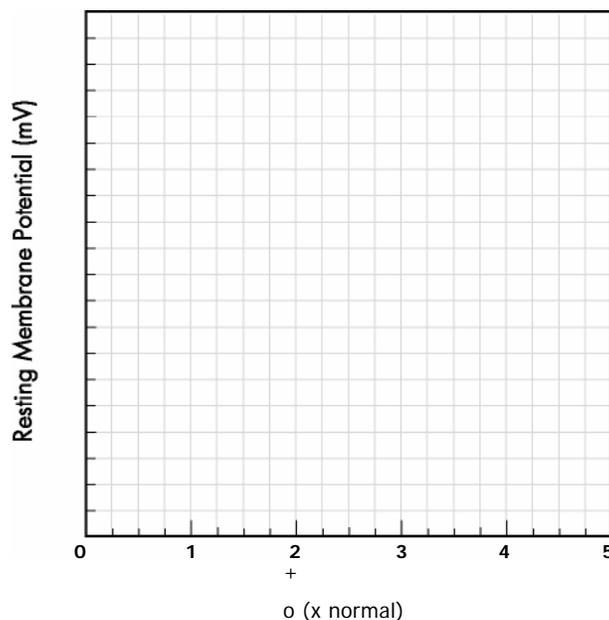
Capacitance refers to the ability of the membrane to store charge without building up a large voltage. If the capacitance is large one must move more charge across the membrane to get the same membrane voltage change. Use this concept to try to explain your results:

Resting Membrane Potential

Vary the external potassium concentration, K^+_o from 0.1 to 5 X normal (to do this choose Environment from the Set Parameters pop-up menu) and measure the resting membrane potential for each value (use the Zoom and Cursor features to get accurate values). Record the data in the table:

Membrane Potential - External K^+

K^+_o (x normal)	Resting Membrane Potential (mV)
0.1	
0.2	
0.5	
1.0	
2.0	
5.0	



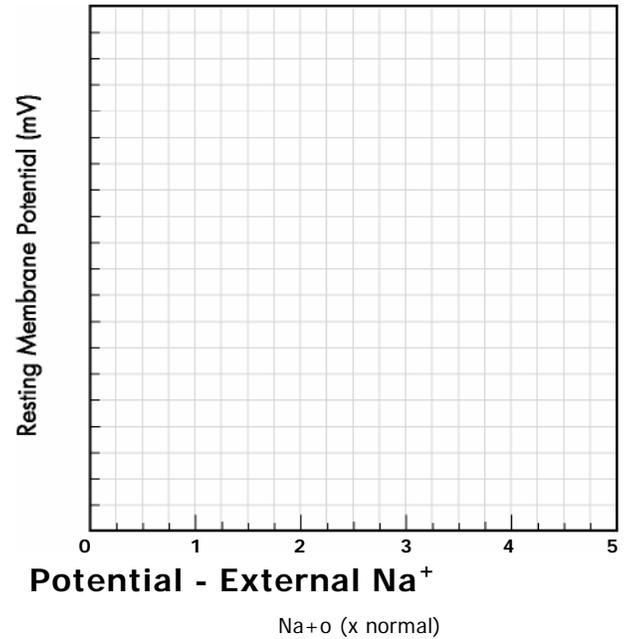
How many millivolts does the membrane potential change for a 10x change in K^+_o ? _____ Although the membrane potential is sensitive to K^+ , the magnitude of the change is somewhat smaller than the 58 mV per decade predicted for a membrane permeable only to K^+ ions (this is the value given by the Nernst equation for 18.5 °C). The reason for the smaller change is that this membrane is fairly permeable to Na^+ and to other ions lumped together as a "leak". These ions also contribute to the potential, lowering the magnitude of the change.



Now repeat the same experiment using external Na^+ instead of internal K^+ and compare the results

Membrane

Na^+ o (x normal)	Resting Membrane Potential (mV)
0.1	
0.2	
0.5	
1.0	
2.0	
5.0	



For an explanation of the electrical potential across a membrane permeable to only one ion (equilibrium or Nernstian potential) go to the Interpret pop-up menu and choose Equilibrium Potential . As you work through the examples you will see that at equilibrium the electrical forces acting on the ions are exactly balanced by diffusional forces. If an ion cannot penetrate the membrane it cannot come to equilibrium and in general the electrical and diffusional forces acting on it will not be balanced.

If an ion is not at equilibrium across a membrane the concentration gradient represents stored electrical energy and might be thought of as a tiny battery for that ion. If a conductance path is opened across the membrane the stored electrical energy can be tapped for doing work (i.e., for making action potentials).



Experiment 3: Reconstructing the Axon: Voltage Gated Potassium Channels

Overview

A. Membrane Action Potentials

In this experiment we add voltage sensitive K^+ channels. The potassium channel of the squid axon has a single voltage-regulated gate to the bare passive axon. Adding this feature will change the properties of the membrane in significant ways, but it will not result in a functioning axon. Although this is a “thought experiment” it can be approached in the laboratory as well. If the Na^+ channels of a nerve are poisoned with tetrodotoxin the resulting membrane will be very similar to the one studied here. Alternately, it is possible to express the K^+ channel gene in a cell such as the *Xenopus* egg and then study the channels with electrodes.

Task

Testing the Membrane with Voltage-Gated Potassium Channels:

Load the experiment Passive Axon: Only K Gates. This axon is identical to the membrane studied in experiment 2 except that it has voltage-sensitive K^+ channels. Set the Recording Time to 20 msec. Run a simulation at stimulus values of $50 \mu A/cm^2$ and 10 msec. Print the curve and compare it with the ones that you obtained in experiment 2 (leaky axon without any voltage activated channels). The shapes of the curves are very different because you have activated K^+ channels in this experiment. How would you explain your results? For help go to the Interpret pop-up menu and choose Gate Animation. Drag the graph cursor (vertical dashed line) back and forth and use the screen gate animation (described below) to help interpret your results. This will give you a picture of the plasma membrane penetrated by K^+ channels (figure 2). As you move the cursor along the graph, channels will open and close. As you can see the K^+ channels do not open instantaneously when the nerve is stimulated. How many milliseconds after the stimulus do you see the maximum K^+ channel opening? .

Recall

Reminder of Gate animation for K^+ channels

Run the Explain gate animation if you have not done it. (Click on Interpret on the bottom menu and select Explain from the popup list.) The following is a quick summary.

Figure 3.1 shows the gate animation with only the K^+ channels drawn.

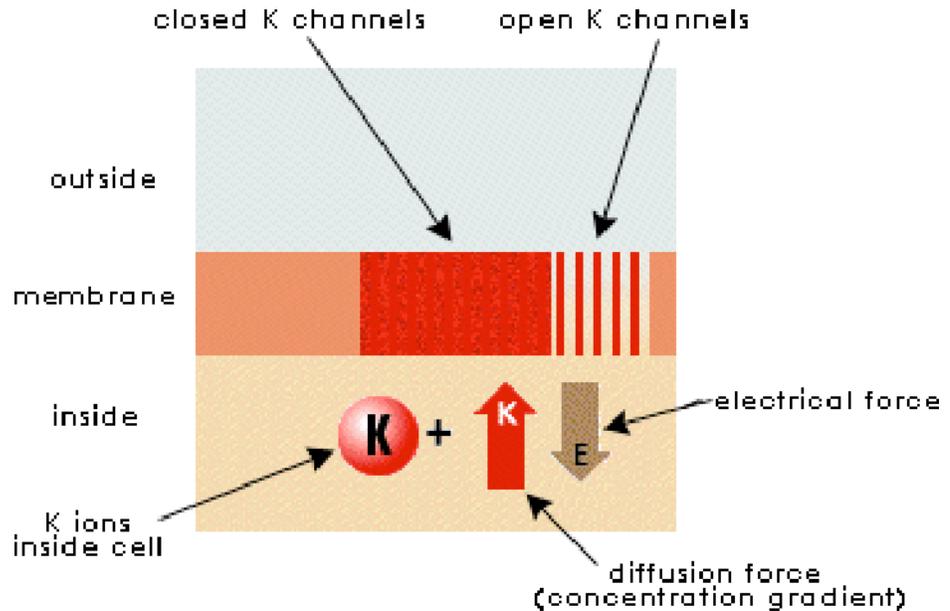


Figure 3.1 Gate animation for K^+ channels

The thick orange-red line represents the axon plasma membrane. The high K^+ concentration is shown on the inside of the cell, poised to exit. There are two arrows associated with the K^+ ion, the red arrow represents the **electrical force** acting on each ion tending to move it across the membrane. The brown arrow representing the **diffusion force** or the tendency of the K^+ ion to diffuse across the membrane, out of the cell, down its concentration gradient. An arrow pointing upwards denotes a force on the ion pushing it out of the cell. A downward arrow pushes into the cell. The net force on the ion is the algebraic sum of the two forces (red "concentration" arrow minus brown "electric" arrow).

There are two requirements for the net flow of an ion through a membrane:

1. There has to be a net force in the direction of flow
2. There has to be an open pathway (or channel) through the membrane.

In Figure 1, open channels are represented by the openings, outlined with red vertical bars, that extend across the membrane. (The number of open channels is proportional to the conductance (g_K) which can be plotted) Each K^+ channel contains a single gate which blocks the K^+ channel when it is closed. Some of these gates are open and some are closed at any particular membrane potential (voltage), but as the voltage becomes more positive, more gates are found in the open position.



Task

Non-Linear K^+ Channels are Responsible for the Undershoot

Look at the trials you have run for this exercise. Note that during a long stimulus, E first rises and then falls to a lower level, even though the stimulus is maintained. This is due to the “sluggish” voltage activated K^+ channels finally opening in response to a depolarization (stimulus causing E to rise and become less negative). This allows K^+ to leave the cell more rapidly, leaving uncompensated negative charge behind. Correlate this response with the opening of K^+ channels shown in the animation. (You may have to use large stimuli for the K^+ channels to show up in the animation). Once activated, the K^+ channels are also slow to turn off when the stimulus is removed. This is apparent in the graph, where E drops slightly below its resting value (negative afterpotential) and only slowly returns as the channels resume their resting configuration. Correlate this with the closing of K^+ channels in the gate animation. This slow return of the K^+ channels to their resting state is related to the refractory period seen in the normal axon. Use dual stimuli to illustrate this. When the two long stimuli are applied close together, the peak response of the second stimulus will be lower than the response to the first.

Overview

B. Measuring the K^+ flow with a voltage clamp

The voltage clamp experiment is not set up to stimulate the axon and observe the resulting membrane voltage (action potential). Instead, you will:

1. Jump the membrane voltage to a preset level
2. Hold it fixed at that level
3. Record the charge delivered to the membrane surface that is required to keep the membrane voltage from changing.

This experimental arrangement, called a voltage clamp, is one of the most valuable techniques in neurophysiology. It allows you to change the voltage and hold it at a chosen value - not letting it run away as it normally does when you stimulate it. This is exactly what is needed to study what happens on depolarization (stimulation). Depolarization is the force which opens or closes gates. You can choose the size of the depolarization and it will stay where you put it, while you monitor the flow of ions (current) through the membrane. Information about specific ions can be obtained by removing some of them from solution or by poisoning certain types of channels. Measurements of ion flow through the membrane can then be used to make inferences about gates opening or closing under your prescribed force (membrane voltage). The interpretation is much simpler in this model where the membrane does not contain any functional Na^+ channels.



Interpret

How the Voltage Clamp Works

To familiarize yourself with the voltage clamp go to the Interpret pop-up menu and choose Voltage Clamp. Run through the series of diagrams which illustrate the voltage clamp applied to a K^+ channel. It is summarized below:

At rest the concentration gradient (red arrow figure 3.2) causing K^+ to diffuse out of the cell is through K^+ channels (outlined by red vertical bars) is nearly balanced by the electrical force (membrane voltage - brown arrow) acting in the opposite direction. As a result very little K^+ leaks out.

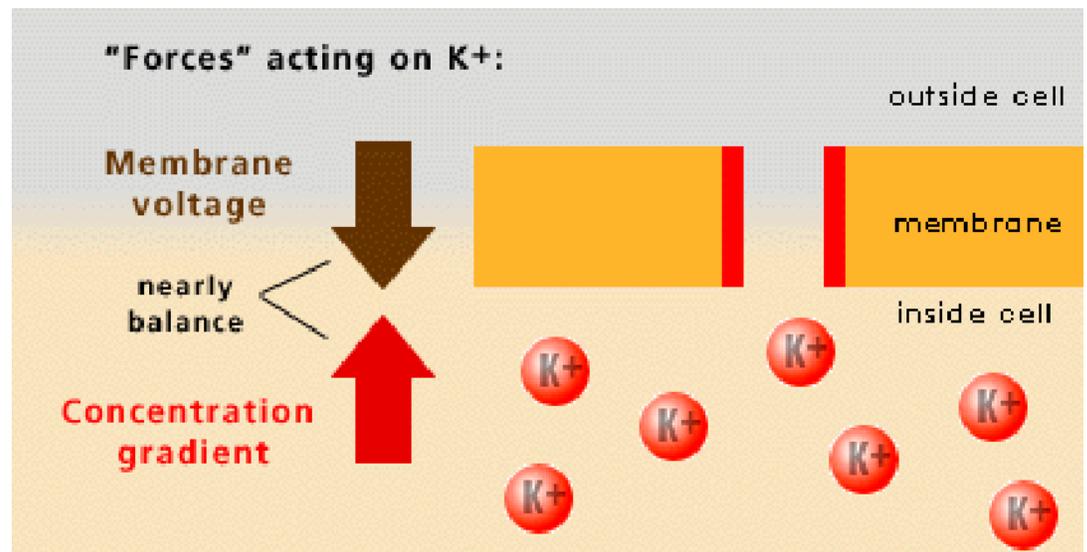


Figure 3.2 The membrane at rest. Arrows show the forces acting on K^+ ions.

When the voltage clamp is turned on, a small pulse of negative charge is delivered to the external membrane surface (an equivalent positive charge is also delivered to the internal surface). This new charge is just sufficient to jump the membrane potential from -65 mV to say -20 mV. See Figure 3.3, below

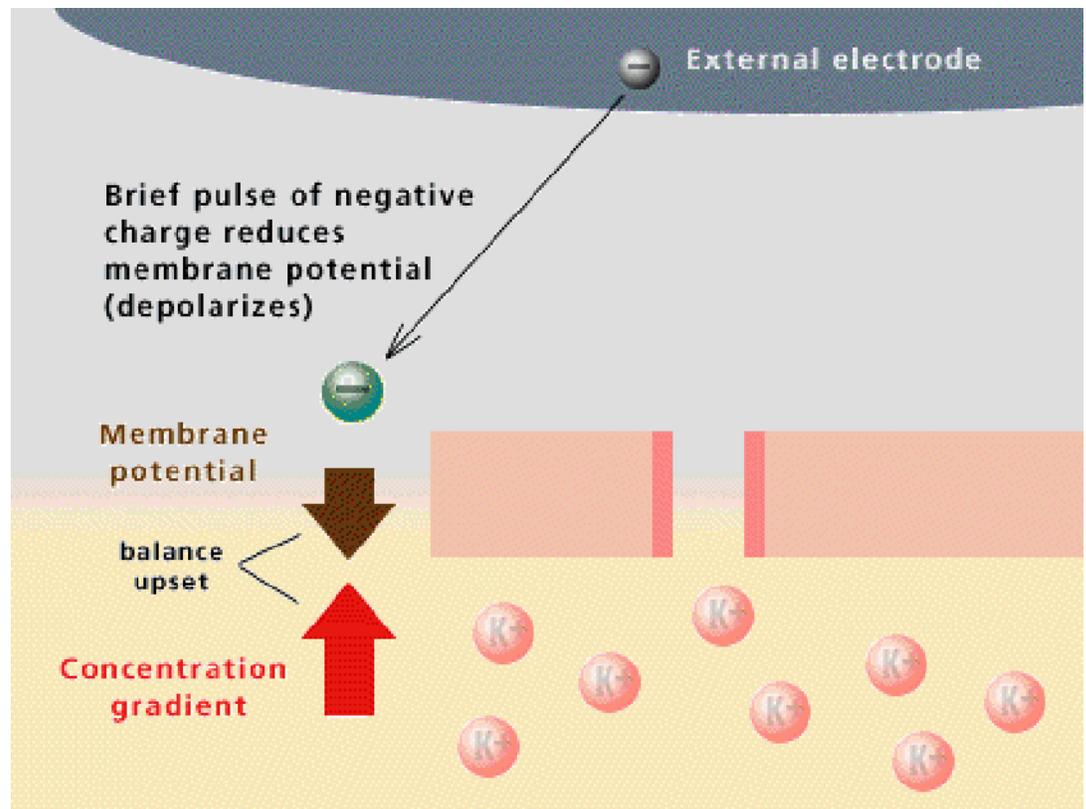


Figure 3.3. Voltage clamp is turned on

This new membrane potential tending to force positive charge into the cell is too small to balance the tendency of K^+ to diffuse out of the cell. In addition, the membrane depolarization opens more K^+ channels (Figure 3.4).

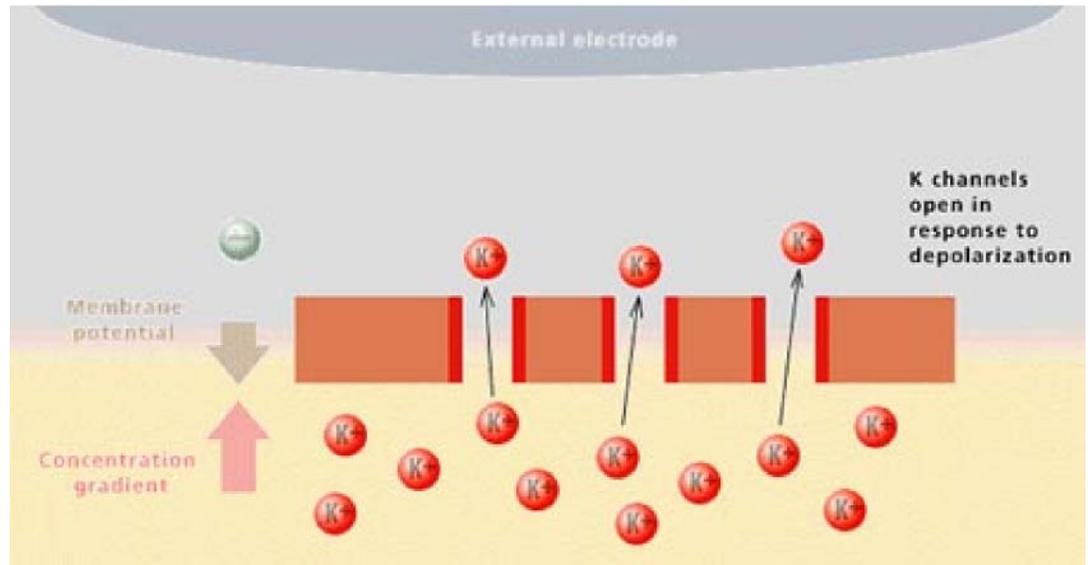


Figure 3.4. K^+ channels open and K^+ flows from cell.

K^+ diffusing out of the cell would add positive charge to the outside and change the membrane potential. However, the voltage clamp monitors E and prevents any change by adding one negative charge for each K^+ that crosses the membrane to leave the cell, see Figure 3.5.

1 The negative charge added to the solution is an ion not a bare electron. The identity of the ion depends on the type of electrode and need not concern us. We have not shown the intracellular electrode which acts in a similar way by "absorbing" the excess negative charges (ions) left behind by K^+ when it moves to the outside

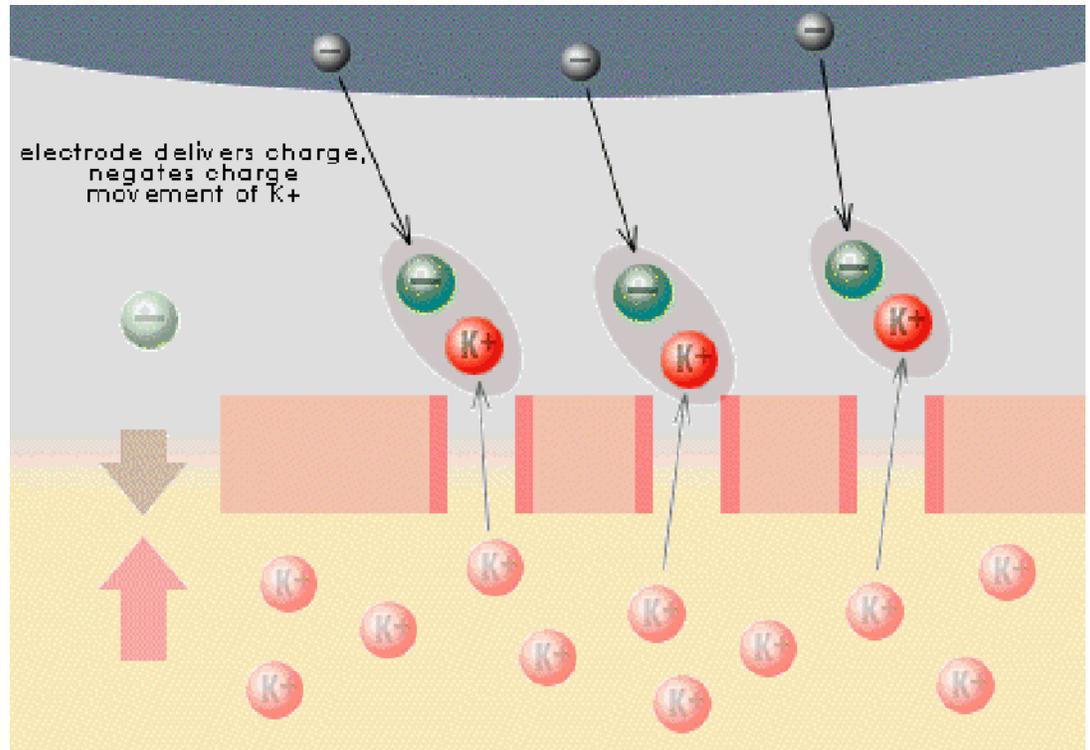


Figure 3.5. Electrode compensates for K^+ flux by delivering negative charge to outside .

Thus, the compensating current delivered by the external electrode is a precise measure of the K^+ leaving the cell. The value of the voltage clamp is due to the fact that even with modern technology it is not possible to chemically measure the small amounts of K^+ that enter or leave the cell within a fraction of a millisecond, but the charge delivered by the voltage clamp can be measured routinely.

Task

Running the Voltage Clamp

Open Voltage Clamp: Only K Gates. Notice that the interface is modified. In previous experiments you began on the left with a current source that we called the Stimulator. You set the parameters (intensity and duration) for the stimulus current and recorded the resulting action potential with the Voltage Recorder on the right hand side of the screen. You measured what happens to the membrane voltage when you imposed a given pattern (in our case a "square wave") of current. In the voltage clamp we reverse the process. We insist on a prescribed voltage and measure what we have to do to insure that the membrane responds accordingly. Instead of "tossing in " some charge and watching what the membrane does, we attempt to control the membrane's behavior.

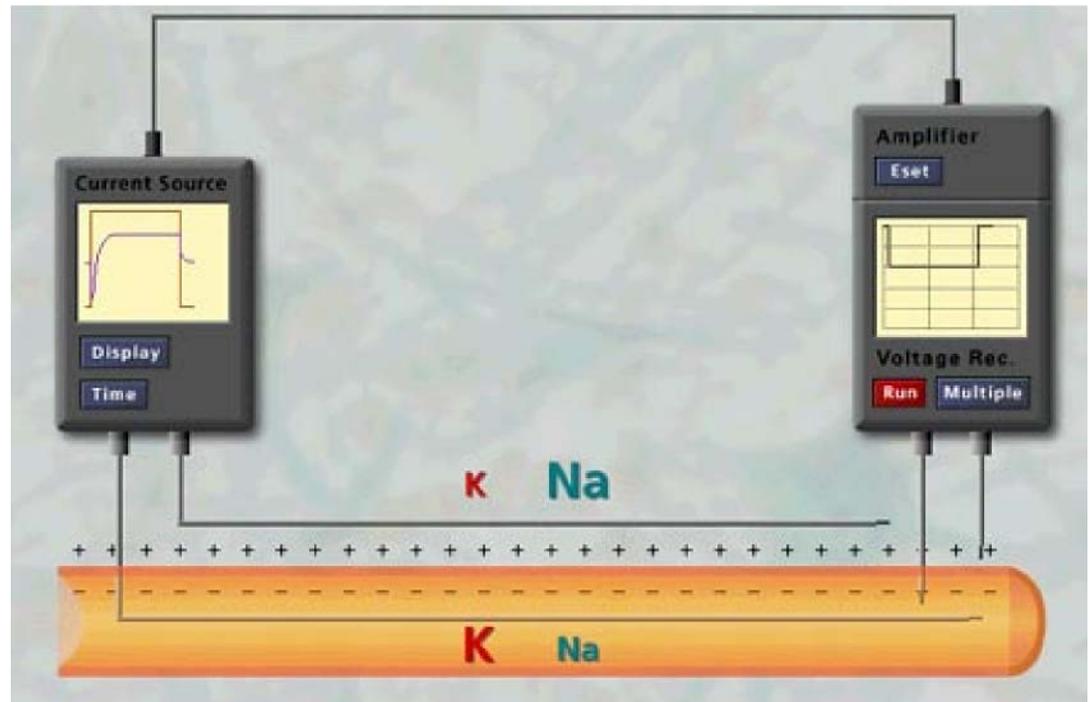


Figure 3.6. Voltage clamp interface

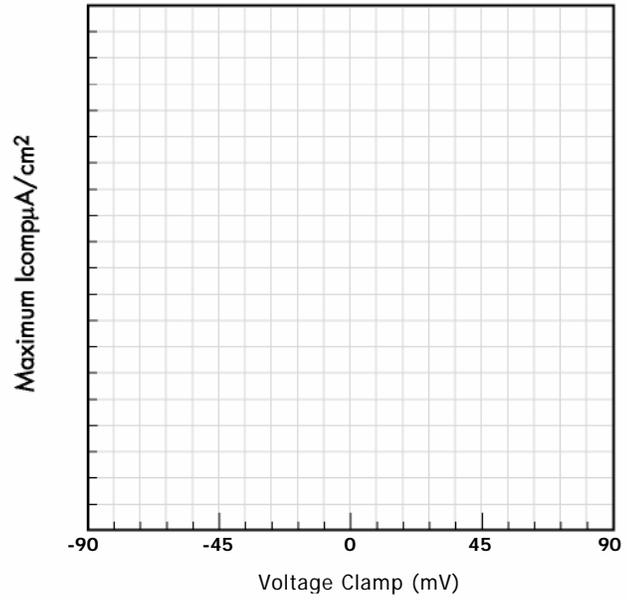
Control usually implies “feedback” and the voltage clamp is no exception. We begin on the right hand side where the Voltage Recorder/Amplifier monitors the membrane behavior (voltage), and sends signals through the wire on top of the diagram back to the current source. These signals instruct the current source on how much electrical charge to feed back to the membrane to insure that the potential does not stray from the desired level which we have prescribed with the Eset button on the amplifier.

Run the simulation with the default settings. The default Eset will begin the clamp at 0.5 msec by “jumping” the membrane potential from -65 mV to -20 mV and will keep it at this value for 10 msec. Drag the Cursor to different positions and use the Gate Animation to see how the K^+ gates open in response to a sudden depolarization. As you will see, they respond slowly, taking about ____-msec for 50% opening. Note that the K^+ channels do not close as long as the voltage clamp is maintained. Compare this behavior with that of the Na^+ channels in the next experiment.

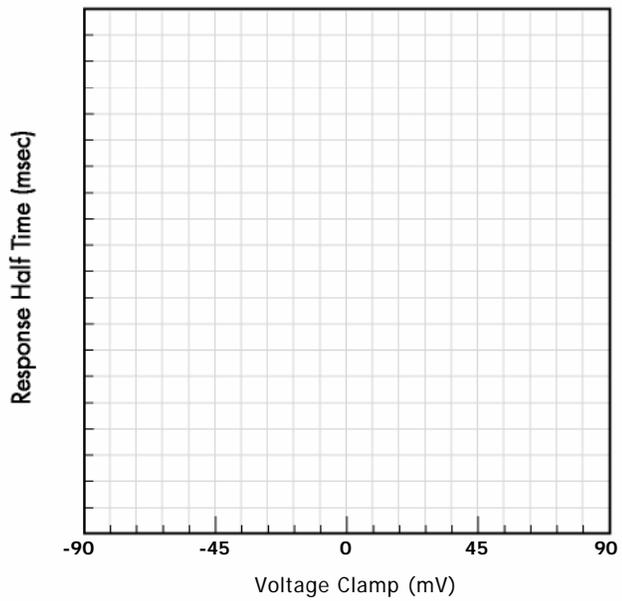
The graph has a plot of I_{comp} , the current the electrode must supply to compensate for ion movements across the membrane. Because there are no Na^+ channels in this membrane, I_{comp} is essentially equal to the K^+ current across the membrane, I_K . It should not be surprising that the time required for I_{comp} to reach 50% of its maximum value is the same as the time required for 50% of the K^+ channels to open. The only way for I_{comp} to change is by changing the number of open K^+ channels because the K^+ concentrations do not change within the short time of the clamp.



Steady State IK - Membrane Polarization

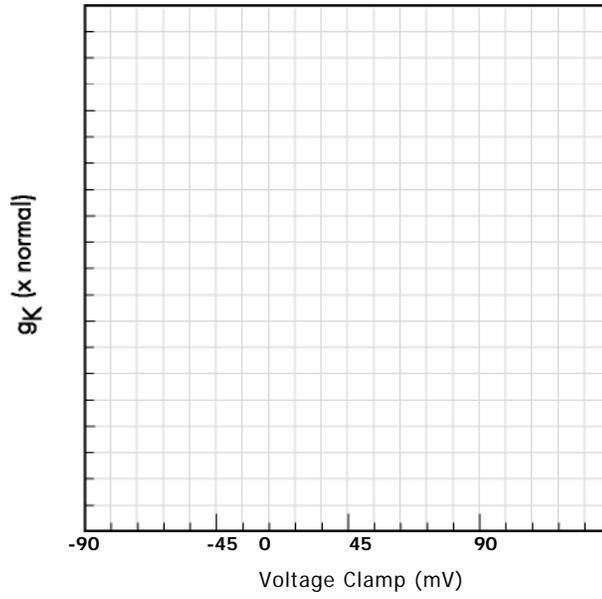


Response Time - Membrane Polarization





K⁺ Conductance - Membrane Polarization





A. Membrane Action Potentials Blocking the

Overview | K⁺ Channels

In this experiment we look at the Na⁺ channels in a membrane in the absence of K⁺ channels. This can be accomplished by inhibiting the K⁺ channels with tetraethyl ammonium ion, or by expressing the Na⁺ channel gene in a cell such as the *Xenopus* egg. The Na⁺ channel differs from the K⁺ channel in that it has 2 “gates”. The gates are referred to as the “fast gate” and the “slow gate”, which describes their rates of opening and closing.

Maintaining the Resting Potential

Loss of K⁺ channels presents a problem because a high K⁺ permeability is required to maintain the resting potential. If the axon is depolarized at rest, many slow Na⁺ gates will be closed. There is no possibility of reopening them by further depolarization and excitation will not occur. To circumvent this, in this experiment the potential is forced to remain at its normal rest value of about -65 mV until ready for stimulation. (Experimentally this can be accomplished with a voltage clamp)

Testing the Membrane With Voltage-Gated Sodium Channels Stimulate this axon and compare it with the results in Experiment 3 (axon with K⁺ channels, but without any voltage activated Na⁺ channels). Record your data in the table below and print a representative response curve.

Response of Na⁺ Channels to Brief Stimulation

Stimulus Intensity μA/cm ²	Response Peak (mV)	Afterpotential Minimum (mV)
50		
100		
150		
200		

As you can see, excitation occurs, but the membrane potential does not return to the normal resting potential. Is the response all-or-nothing? Can the membrane be excited at its new more positive membrane potential? Test by giving a second stimulus a few milliseconds after the peak. Is the second response all-or-nothing?



After the excitation has subsided the axon remains depolarized; most fast gates are open but the slow gates are closed. Use the Gate Animation (described below) and drag the graph cursor (vertical dashed line) back and forth and to help interpret your results. Na^+ channels differ from K^+ channels; when the membrane is stimulated, the Na^+ channels open very quickly, and remain open only for a very short time. You can also see this by plotting g_{Na} , the Na^+ conductance, which is proportional to the number of open Na^+ channels. This is interpreted in terms of two voltage activated gates controlling the Na^+ channel.

Note: Many axons have both Na^+ and K^+ channels, but in some the Na^+ channels are the only ones that are voltage activated (i.e. have gates that respond to depolarizing the membrane). In these cases opening of the fast Na^+ gates is again responsible for the rise of the action potential, closing of the slow Na^+ gates is sufficient to begin the repolarizing phase, and the abundance of continuously open K^+ channels is sufficient to return the potential to an operative resting level

Reminder

Gate Animation for Na^+ Channels

Run the Explain gate animation if you have not done it. (Click on Interpret on the bottom menu and select Explain from the popup list.) The following is a quick summary.

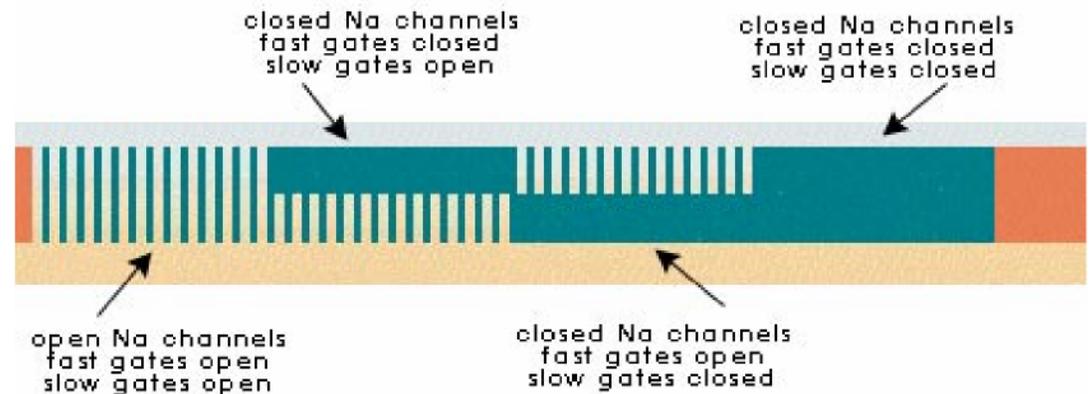


Figure 7 Membrane gate animation with only Na^+ channels.

The continuous paths through the membrane represent open channels. The more paths, the more channels. (The number of continuous paths is proportional to the conductance (g_{Na}) which can be plotted). For K^+ there is either a full path or no path corresponding to one gate open or closed (see Exercise #3). For Na^+ it is more complicated – there are two gates. The slow gates are on the inner (bottom) side of the membrane. When they are open they contribute only half a path. The fast gates are represented on the outer (top) side of the membrane; they also contribute one half path. For Na^+ to pass, you need a full path – both gates must be open.



Overview

Task

The Na⁺ gates are illustrated further in the following diagrams where it can be seen that any given channel has 4 possible states:

State	1	2	3	4
Fast Gate	closed	open	open	closed
Slow Gate	open	open	closed	closed

State 2 is the only open channel which permits passage of Na⁺; all others (States 1, 3, and 4) are impermeable.

Follow the gate animation as you drag the graph cursor.

B- Measuring Na⁺ flow with a voltage clamp

The voltage clamp concept is explained in Experiment 3B and in the Interpret: Explain Voltage Clamp. This experiment follows a similar routine to Experiment 3B, but now we continue with an axon that has its K⁺ channels blocked, and we monitor the response of Na⁺ channels to sustained depolarizations. The results illustrate the experimental evidence that upon depolarization the Na⁺ channels are first activated (i.e. they open) and then they close, even though the depolarization is maintained.

Run the Explain Voltage Clamp in the Interpret popup menu if you have not done it.

Interpret I_{comp}

Open Voltage Clamp: Only Na Gates. The default E_{set} is set to begin the clamp at 0.5 msec by “jumping” the membrane potential E from -65 mV to -20 mV and keeping it at this value for 10 msec. Run the simulation and remember that:

1. There are no activated K⁺ channels. Therefore, for most depolarizations, ion flow through the membrane is practically all due to Na⁺ ions. In this case I_{comp} is equal to I_{Na}
2. The membrane potential is clamped at a given value and does not change. Since the Na⁺ concentrations also do not change within the short time (10 msec) of the clamp, it follows that none of the driving forces for ion flow change. The only way for I_{comp} to change is by a change in the number of open Na⁺ channels.

Notice that in response to a sustained depolarization, the I_{comp} (which is now equal to the Na⁺ current) first rises and then falls- even though the depolarization is maintained. Print the result



Interpretation

Na⁺ channels first open and then close because of the operation of a fast and a slow gate within each channel. At rest most fast gates are closed, but slow gates are open. Upon depolarization, first the fast gates open creating a number of open channels (both gates open). Then the slow gates close shutting down these channels. To follow this, click on the Display button and open the Interpret: Gate Animation. Drag the graph cursor (vertical dashed line) to different positions and use the cartoon to see how the Na⁺ gates open and close in response to a sudden depolarization.

Task

You can also see the dynamic properties of the channel plotted directly on the display. Click on the g_{Na} button to plot the Na⁺ conductance which is proportional to the number of open Na⁺ channels. Now make room for other plots by turning the g_{Na} off; simply click the g_{Na} button once more. Click the pNaf and pNas buttons. This will plot the fraction of open fast gates (probability of finding an open fast gate) and the fraction of open slow gates respectively. These fractions (probabilities) range from 0 (all closed) to 1 (all open). Compare the response times for the opening of the fast gates and the closing of the slow gates.

With a single clamp, use the Slider to run a few different experiments with different values of the clamped voltage (intensity1), within the range of say, -90 mV to + 90 mV. Note that some I_{comp} will be negative (Na⁺ flowing into the cell) while others will be positive (Na⁺ flowing out of the cell) Assuming $I_{Na} = I_{comp}$, find the membrane potential (clamped voltage, intensity1) where maximum $I_{Na} = 0$ (use interpolation to “zero in” on the correct value). This is the **equilibrium potential** for Na⁺. It can be calculated from the internal and external Na⁺ concentrations by use of the Nernst equation Membrane potentials above this value will generate positive currents sending Na⁺ out of the axon, while potentials below this value generate negative currents sending Na⁺ into the axon.

Na⁺ conductance g_{Na} reflects number of open Na⁺ channels

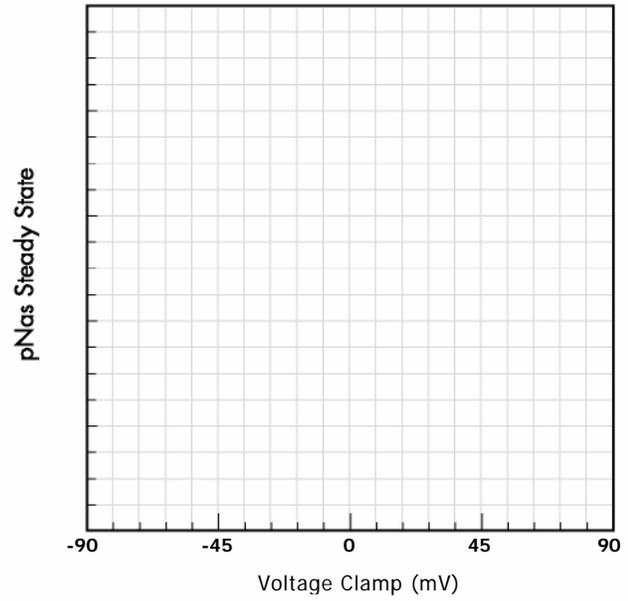
The Na⁺ conductance g_{Na} is a more useful indicator of the channel behavior because it is proportional to the number of open Na⁺ channels, and unlike, I_{comp} (or I_{Na}), it is always positive. Although g_{Na} is not directly measurable, it is easily calculated¹ from I_{comp} . The computer carries out this computation for us and you can toggle it on and off the display by clicking on the g_{Na} button . Using the display buttons turn I_{comp} off and g_{Na} on.

¹By definition $g_{Na} = I_{Na} / (E - E_{Na})$. In our case $I_{Na} = I_{comp}$, and $E = E_{set}$ so that

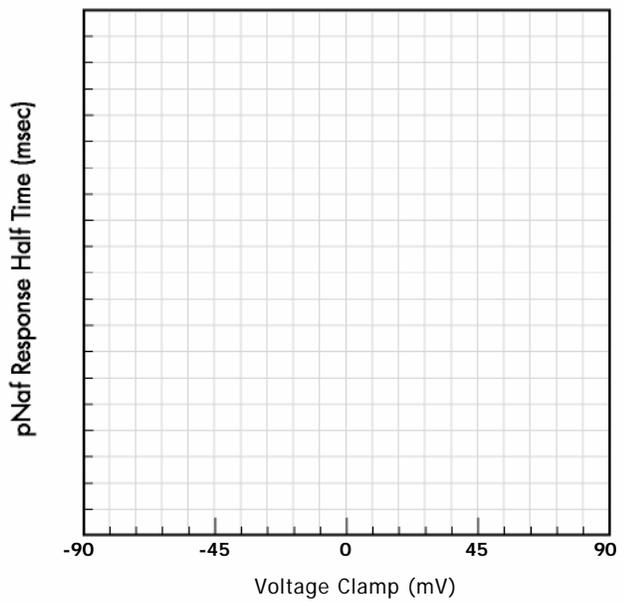
$g_{Na} = I_{comp} / (E_{set} - E_{Na})$.



Slow Gate Open (Steady State) - E

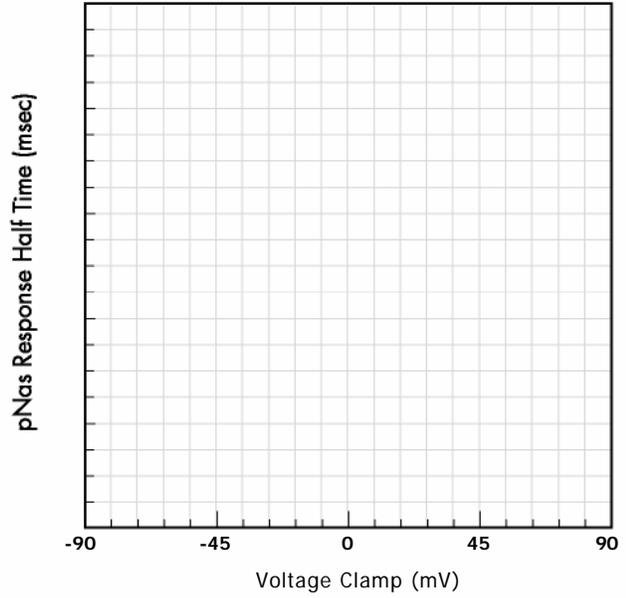


Fast Gate Response Time - E

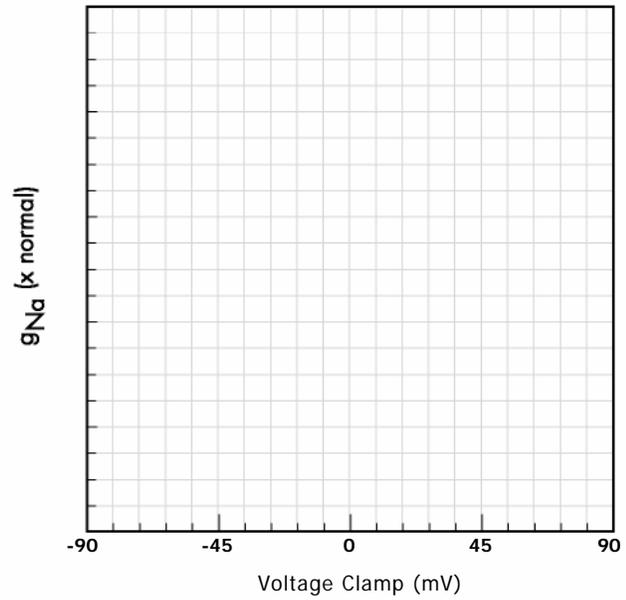




Slow Gate Response Time - E



Peak Na+ Conductance - E





Note that when p_{Na} increases, p_{K} decreases and vice versa. Compare the response time curves with the K^+ gate response time observed in exercise 3. Taken together these, curves yield a comprehensive characterization of the ion channels.

Refractory Properties

Further insight into the refractory period can be obtained from experiments which use two clamps. To prepare for dual clamps, click on the time button on the Current Source and increase the Recording Time to 24 msec, and set up the following pattern of clamps by clicking on Eset:

Clamp1

onset (msec)	0.5
intensity (mV)	-20.0
duration (msec)	10.0

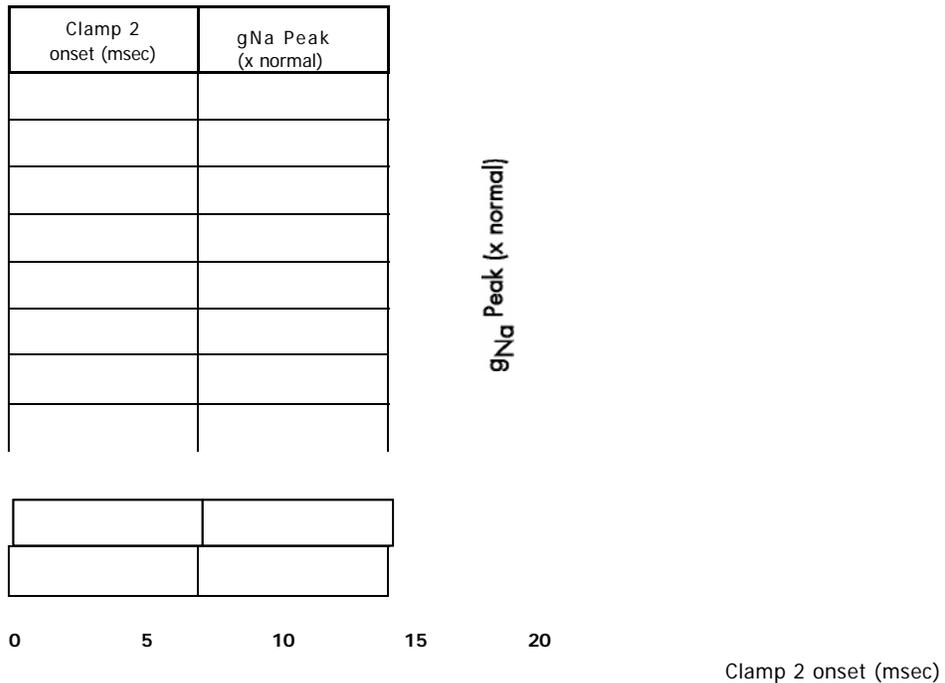
Clamp2

onset (msec)	11.0
intensity (mV)	-20.0
duration (msec)	10.0

This provides a depolarization to -20 mV, with sufficient time for the channels to respond fully to the new membrane potential. A "rest period" of 0.5 msec follows and then depolarization is repeated. Run the simulation. Turn g_{Na}^{on} and turn any other variables off the Display. It is apparent that the "rest period" was not sufficient. The response to the second stimulus much smaller than the response to the first. This is because most of the slow gates have closed in response to the depolarization, and 0.5 msec is not long enough for them to reopen. The channels are "deactivated" and are not able to open in response another stimulus. Run the Gate Animation to see this. How long will it take for the channels to recover? Using the Slider to vary Clamp 2 onset, run 10 runs ranging from 11 to 20. This shows the gradual recovery of the channels as the slow gates reopen. Plot the peak of g_{Na} versus Clamp 2 onset (i.e. the "recovery period").



Dual Clamps



Channel Response to Depolarization Depends on the Starting Point

You may have noticed in the Gate Animation that at rest, when $E = -66$ mV, many of the slow gates are closed. We have already seen that when we depolarize the membrane more of the slow gates close. This raises the question of whether we can force more gates to open by going in the opposite direction i.e. hyperpolarize by making E more negative than -66 mV. To test this set up the following pattern of clamps for a control:

Clamp1

onset (msec)	0.5
intensity (mV)	-66.0
duration (msec)	10.0

Clamp2

onset (msec)	11 .0
intensity (mV)	-20.0
duration (msec)	10.0



Clamp 1 holds the potential to near its resting value and then Clamp2 tests it with a standard depolarizing clamp. Run the simulation with g_{Na} on, and there should be no surprises; it looks like our first record. Now we test what happens if the "holding potential" (i.e. intensity1) is made more negative. To prepare for the comparison activate the O button (overlay) on the Display so that the screen will not be erased when we initiate the second run. Set up the clamps for the experimental run as:

Clamp1

onset (msec)	0.5
intensity (mV)	-90.0
duration (msec)	10.0

Clamp2

onset (msec)	11 .0
intensity (mV)	-20.0
duration (msec)	10.0

Run the simulation and compare the g_{Na} , I_{comp} , etc. in the two cases. Print the graphs. Check out the Gate Animation to help interpret your result. This result is generally true. Most ion gates are only partially activated (or deactivated) at resting potential. It allows the system to move in both directions and has physiological implications.

**Overview****A - Membrane Action Potentials**

Finally, we reconsider the normal axon with a full complement of voltage activated Na^+ and K^+ channels. The temperature is lowered to 6°C to slow things down and make them easier to track. Our objective will be to interpret the qualitative properties of the axon (e.g. threshold, refractory period, all or none response) in terms of the action of the channel gates.

Task

Open Membrane Action Potential: Advanced.. Open the Environment dialog from the Set Parameters popup menu, and change the Temperature to 6° . Run the simulation. Analyze your results using the gate animation with the Graph cursor as well as plots obtainable from the variable buttons on the graph. In interpreting these data, remember that the amount of charge movement necessary to make substantial changes in E is very small. During the short time of a single action potential, the actual amounts of Na^+ and K^+ that move in or out of the axon are very small; they have significant effects on E , but the concentrations of Na^+ and K^+ hardly change. This is illustrated in the program – the concentration arrows do not change during the action potential (drag the graph cursor back and forth) even though the E arrow does.

Recall

Recall that a stimulus causes a brief increase in the number of open Na^+ channels. If the stimulus is weak, only a few channels open and the membrane is hardly perturbed. However if the stimulus is sufficiently strong, i.e. if it is stronger than a critical level called the **threshold**, then the number of open Na^+ channels becomes very substantial. Na^+ ions, poised at high concentration outside the axon, leave their negatively charged “partners” behind on the outside and rush in fast enough to overwhelm the K^+ moving out. The inside of the cell is inundated with positive charge so that the polarity is reversed; now the inside is positive and the outside negative. A moment later the Na^+ channels close and extra K^+ channels open. The membrane becomes very permeable to K^+ . K^+ moves out making the membrane potential even more negative than it was at rest, driving it very close to the K^+ equilibrium potential. Finally (after several msec) the extra K^+ channels close and the membrane returns to its resting condition.

Task**Ion Currents**

To see the inflow and outflow of ions, add the sodium current i_{Na} and the potassium current i_{K} to your plot (access these by using variable buttons on the graph). **By convention outward flow of positive ions (K^+ in our case) is positive, and inward flow (Na^+ ions) is negative.** (Make sure that all currents are scaled on the right hand axis; otherwise it will dwarf E . You can switch Y axes by holding the shift key while clicking on the variable button.). You can also plot the net current i_{sum} to see the net flow of positive charge. This will consist almost entirely of i_{K} and i_{Na} . (The only other flow of charge is called the leakage current i_{L} . Try plotting it to show that it is negligible



during excitation.) Note how small the stimulating current is compared to the sodium or potassium currents, or even the net current. It gives some indication that the axon is "loaded" with its own energy source (ion gradients that have been set up by the Na⁺-K⁺ pump) and ready to be triggered by a small disturbance. (In making this comparison, be sure to scale the Stim and other currents on the same Y axis.) It is now apparent that a relatively small current stimulus evokes an enormous response of membrane currents, emphasizing the explosive nature of the excitatory process.

Rate of change of E is proportional to -I_{sum}¹

When I_{sum} is negative (positive charge flows into the cell) E will increase, when it is positive E will decrease. Verify this by moving the graph cursor to the point where I_{sum} just crosses the zero line and changes from negative (positive charge flowing in) to positive (positive charge flowing out). At this moment E has reached its maximum value, moments before it was increasing, moments later it will decrease. In fact, the rate of change of E is proportional to -I_{sum}. You can verify this by picking off the slope (rate of change) of the action potential at convenient points and plotting the slope versus I_{sum}. To compute an approximate slope you need two points on the curve that fairly close together (say no more than 5 mV apart). Denote these two points by E1 and E2 and their corresponding times as t1 and t2. The slope at the point midway between E1 and E2 will be approximately

slope (E2 - E1) / (t2 - t1)

Try it for at least 3 points. When you plot the slope versus I_{sum}, you should get a straight line.

E	Slope of action potential	I _{sum}
Crossing zero going up		
peak	0	
Crossing zero coming down		

¹This is only true for the membrane action potential. When the action potential is propagated, currents originating from up and downstream portions of the axon act like an external electrode and also contribute to the rate of change of E.

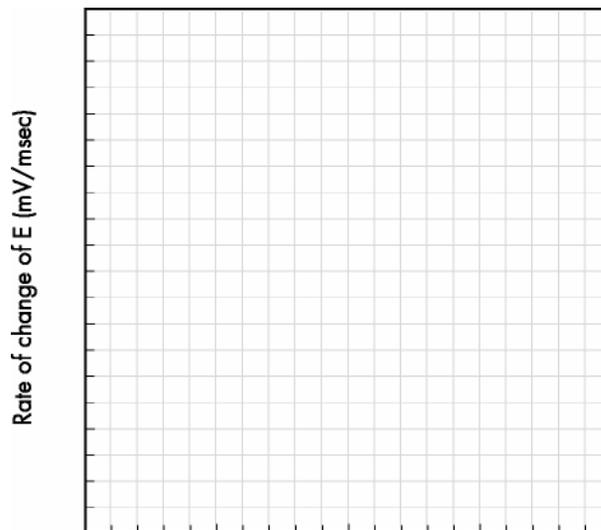


Rate of Change of E - Isum

E (mV)	Rate of change of E (mV/msec)	Isum ($\mu\text{A}/\text{cm}^2$)
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Opening and closing of gates depends on E

The behavior of the axon is governed almost entirely by the precise timing of Na^+ and K^+ gates as they open and/or close in response to changes in mem-



brane potential (E). The gate animation gives a visual picture of the position of these gates at any particular time. The following table (Figure 5.1) is a simple qualitative reminder about how these gates respond to changes in E (membrane potential)

Recall



Gates	Speed of Response	As E increases (becomes more positive)	As E decreases (becomes more negative)
K	Slow	Opens	Closes
Fast Na	Fast	Opens	Closes
Slow Na	Slow	Closes	Opens

Figure 5.1

Task

Move the graph cursor around and try to interpret the results remembering that:

1. Changes in E reflect changes in net charge on the inner surface of the membrane.
2. Increases in net charge are promoted primarily by positively charged Na^+ ions moving into the cell and opposed by positively charged K^+ ions moving out.
3. Movements of Na^+ or K^+ are determined by the net force on the ion as well as the number of channels that are open to that ion.
4. Both fast and slow Na^+ gates must be open in the same channel in order for the channel to be open.

Clean up the display

More precise information about channels and gates can be obtained directly from the plot. To prepare for this, clean up the display by turning off all the plotted variables (e.g. E, Stim, ENa, EK, as well as any of the currents I_{Na} , I_{K} , I_{L} , I_{sum}) The display should be empty and all of the variable buttons will be gray.

The number of open channels

Now turn on gK. gK plots the potassium conductance which is proportional to the number of K^+ channels that are open at any time. Verify this by running the graph cursor across the plot and notice the correspondence: K^+ channels open as gK rises, and they close as gK falls. Turn on E. Both E and gK are plotted on the same axis so that the scale changes; gK appears smaller in order to accommodate the larger magnitude of E. You can restore the size of gK by holding down the shift key and clicking on the gK button. Try it. gK is now scaled on the right hand axis and is restored to its original size. Again, hold down the shift key and click on gK. Scaling returns to the right hand axis. This will work for any variable; to change its scaling from one axis to the other, simply hold down the shift key and click on the variable's button.

Turn off gK and E, and turn on gNa. gNa plots the sodium conductance which is proportional to the number of Na^+ channels that are open at any time. Run the graph cursor across the plot and verify the correspondence between open Na^+ channels and g_{Na} . Now turn on both E and gK. This plot is a useful summary relating the opening and closing of the ion channels to the action potential at each moment of time. Print your results.

Probability of finding an open gate

Details of the Na^+ channel states can be also be plotted. Turn off gK and turn on pNaf. pNaf plots the fraction (probability) of fast gates that are open at any time.



Again, run the graph cursor across the plot and notice the correspondence: fast gates open as p_{Na_f} rises, and they close as p_{Na_f} falls. Turn p_{Na_f} off and turn on p_{Na_s} . This will plot the fraction (probability) of slow gates that are open at any time. As you run the graph cursor across the plot notice how the slow gates close and open as p_{Na_s} falls and rises. Now turn off E and turn on g_{Na} . This plot shows how the opening of Na^+ channels, g_{Na} , is determined by the actions of the two gates. Actually g_{Na} is proportional to the product $p_{Na_f} \times p_{Na_s}$. Print the plot.

The same exercise can be repeated for p_K , the fraction of K^+ gates that are open. But since K^+ channels only have one gate there is a one to one correspondence between open K^+ channels and open K^+ gates. p_K gives no more information than g_K .

Recall

1. **Threshold** If a nerve is stimulated with weak electrical shocks, nothing seems to happen. When the stimulus is repeated many times, with each stimulus a little stronger than the last, eventually a point will be reached where the nerve responds by transmitting an action potential. The strength of stimulus just barely able to excite is called the threshold. Stimuli below threshold do not work, stimuli above threshold produce action potentials.

2. **All-or None** A stimulus above threshold excites, but the size of the response is independent of the strength of stimulus. All action potentials are the same no matter how large the stimulus; the response is all-or-none. This behavior is similar to a fuse; once lit, the size of the spark that travels along is independent of the size of the match that initiated it.

3. **Refractory Period** The recovery period following excitation when the axon appears to be inexcitable is called the refractory period.

4. **Negative Afterpotential** During an action potential E quickly rises to a positive maximum, then falls below its negative resting potential and then slowly rises back to its resting potential as it fully recovers. This final portion of the action potential where E is below the resting potential is called the negative afterpotential.

Interpret

To interpret these properties, recall that the inside of the axon has high K^+ , the outside high Na^+ . Further, the membrane potential is a measure of the electrical force on a positive charge. Finally, remember that the amount of charge movement necessary to make substantial changes in E is very small. During the short time of a single action potential, the actual amounts of Na^+ and K^+ that move in or out of the axon are very small; they have significant effects on E , but the concentrations of Na^+ and K^+ hardly change.

At rest the axon is permeable mostly to K^+ , but not much K^+ leaks out because the opposing membrane potential, E , is close to the K^+ equilibrium potential



(i.e. the concentration gradient of K^+ is almost balanced by E pushing in the opposite direction).

At rest, the membrane is polarized. Many slow gates are open but most rapid gates are closed so that most channels are closed. When the membrane is stimulated, its response to the depolarization can be divided into 3 time phases:

1. An **early response** when the rapid gates open quickly. **Na^+ channels open**. Now both gates are open so that channels become freely permeable to Na^+ , and Na^+ rushes into the axon causing E to rise in the positive direction.
2. A **late response**. A moment later the slow **Na^+ gate closes**. The membrane is no longer highly permeable to Na^+ , the rapid inflow of Na^+ ceases. In addition the slowly responding gates in the **K^+ channel open** and K^+ flows out of the axon, causing E to fall in the negative direction.
3. A **recovery phase**. Still later, all gates return to their original resting position.

With a weak **sub threshold** stimulus, not enough Na^+ flows in to overcome the outflow of K^+ and the axon repolarizes.

With a stronger, **supra threshold** stimulus, more Na^+ channels open so that Na^+ inflow exceeds K^+ outflow, the net flow of charge is now positive inward and the axon is depolarized even further. But this opens even more Na^+ channels which causes more depolarization. A vicious cycle ensues; the membrane potential takes off in the positive direction with an explosive velocity as the interior of the axon becomes more and more positive. But this rapid upward movement of the membrane potential does not persist. Soon E becomes positive and large enough to oppose Na^+ entry despite the open channels, i.e. E approaches the Na^+ equilibrium potential (where the concentration gradient moving Na^+ inward is just balanced by E pushing Na^+ out). At the same time the delayed effects begin to appear. Na^+ channels close and voltage activated K^+ channels open, K^+ outflow exceeds Na^+ inflow, and the net flow of charge is now positive outward. E plummets toward its resting value, overshoots momentarily and comes very close to the K^+ equilibrium potential because the voltage activated K^+ channels are still open making the membrane even more K^+ permeable than it was at rest. Finally the repolarized membrane closes the voltage activated K^+ channels and E returns to its resting value.

**Task**

From this description, we see that the **threshold** is determined by the stimulus strength that is able to cause an inward Na^+ flow to exceed the outward K^+ flow. From that point onward, the stimulus plays no further role because the seeds of the positive feedback (vicious cycle) reside in the axon itself.²

Verify this by plotting I_K and mI_{Na} as well as E on the same graph. mI_{Na} is the same as I_{Na} except that it is plotted in the upward direction (i.e. $mI_{\text{Na}} = -I_{\text{Na}}$). This makes it easier compare it with I_K . To optimize the scaling, Keep E on the left axis and move I_K and mI_{Na} onto the right axis if they are not already there. (You can shift the axis of any variable by holding the shift key down and click on its button.) Find the approximate threshold, and then click on the “fine check box to gain greater control. Now find an accurate threshold. You can do this by clicking on the right hand (or left hand) arrow on the slider to add (or subtract) very small increments to the intensity. Notice that below threshold I_{Na} remains lower than I_K . However when threshold is reached, I_{Na} overtakes I_K . Print a plot of the currents when the stimulus is just barely below threshold and compare it to a print of the plot where the stimulus is just above threshold.

These results follow provided the late response has not had time to develop. In some cases with stimuli that are just under threshold the I_{Na} may momentarily exceed I_K , only to fall back as the late response (K^+ channels open, Na^+ channels close) sets in. To see an example of this, repeat the above experiment with the temperature set at 18° .

Interpret

The **all-or-none** response arises naturally out of this positive feedback; once the response is triggered, the positive feedback drives the membrane potential to its maximum value (near the Na^+ equilibrium potential). The size of the action potential is determined by the concentration gradients of Na^+ and K^+ . The concentration gradient of K^+ limits the resting potential (K^+ equilibrium potential) while the concentration gradient of Na^+ limits the height of the action potential (Na^+ equilibrium potential). Just as a stick of dynamite contains its own explosive energy, the axon membrane is “loaded” with “explosive” energy in the form of ion gradients.

²Although this description captures the essence of the excitation process, the precise position where excitation occurs is a bit more complicated because we have ignored contributions of the leak current. One further qualification is that the process must take place before the late response (K channels open, Na channels close) has time to develop.



For a brief msec or two following excitation, the axon is no longer excitable. This recovery phase, called the **refractory period** can be divided into two phases. The earliest phase is the **absolute refractory period** where the threshold appears to be infinite and no stimulus will suffice. In the later phase, the **relative refractory period**, the threshold returns to normal. The basis for the refractory period is found in the "late effects". After the first msec of excitation the slow Na^+ gates close and remain closed for a brief time despite the fact that E is near rest. These gates were slow to respond to the initial depolarization, and they are equally slow in responding to the repolarized membrane. In addition the voltage activated K^+ gates are still open and this drives E below the resting potential, creating the **negative afterpotential**. With the slow Na^+ gates closed and the K^+ gates open, it is difficult if not impossible for Na^+ inflow to exceed K^+ outflow (i.e. to reach threshold). You can illustrate this by plotting the mINa and IK that result from a second stimulus delivered in the refractory period. However To make this plot intelligible, you may have to use the zoom to see some of the results.

How do the activities of the $\text{Na}^+ - \text{K}^+$ pump influence the action potential? They don't, at least not directly. Any contributions by the pump to is swamped out by the more massive movements of the ions through the channels. The pump does not cycle often enough to make a difference during activity. However, action potentials are very brief and the axon is at rest most of the time. During rest there is ample time for the slow cycling of the pump to restore the small amounts of Na^+ and K^+ that have leaked through channels activated during the action potential.

Task

From your knowledge of the excitation process, complete the following table by predicting (i.e. Increase, decrease, or no change) the effects of changing K^+ or Na^+ ion concentrations on the items listed in the titles to the last four columns.

Predictions

Na^+	g	Na^+ / K^+	K^+	Threshold	Action nels Potential	Negative After- potential	Relative Refractory Period
1	0.1	1	1				
1	1	1	1				
1	0.5	1	1				
5	1	1	1				
1	1	1	0.1				
1	1	0.1	1				
1	1	1	5				
1	1	5	1				

**Overview**

Run the corresponding simulations to verify your predictions. Print the results and provide the reasoning that forms the basis for your predictions.

B - Voltage Clamp Na⁺ and K⁺ Channels

Open Voltage Clamp: Ideal. This simulates a normal axon under voltage clamp conditions. (Familiarity with Experiment 3B is assumed.) Recall that in a voltage clamp experiment you:

1. Jump the membrane voltage to a predetermined level
2. Hold it fixed at that level
3. Record the ion flow through the membrane that is required to keep the membrane voltage from moving.

Task

Run the simulation. While the response of the axon in Exercise 3B was governed exclusively by K⁺ channels, the axon in this simulation has operative Na⁺ channels as well as K⁺ channels.

Look at the gates in the cartoon as you scroll through the simulation (drag the graph cursor). At the start, t=0, with no clamp turned on there are plenty of slow Na⁺ gates open but no fast ones. Now move forward in time, as the clamp is turned on the membrane depolarizes, fast gates open and for some time, the slow gates are also still open; Na⁺ can get through. But, soon, even though the depolarization is maintained the slow gates close and Na⁺ can no longer pass. At the same time open K⁺ channels begin to appear. The membrane will stay in this state as long as the depolarization is maintained.

The experimenter actually measures the net current I_{comp} . Just as in 3A, I_{comp} shows the charge flow required to keep the voltage at the desired value (e.g. -20 mV). But, now this charge is required to balance the flow of both Na⁺ and K⁺. Ignoring the very small contribution of other ions (I_{Leak}), we write

$$I_{\text{comp}} = I_{\text{Na}} + I_{\text{K}}$$

In this simulation we follow the usual convention: a positive I_{comp} corresponds to positive charge moving out of the cell, a negative I_{comp} corresponds to positive charge moving into the cell.

The dip in the curve is negative and it corresponds to Na⁺ entering the cell, while the later rise is due to K⁺ leaving. You can verify this by poisoning the Na⁺ channels: First, click the O (Overlay) button on the Display so that you will have a normal control to compare with the poisoned channels. Next look for nNa, the number of Na⁺ channels, by selecting Membrane from the Set Parameters menu, and set nNa = 0. Run the simulation. The "dip" disappears. Finally, to verify the K⁺ contribution, reset nNa to 1 and set nK = 0 so



Overview

that all of the K^+ channels are poisoned. Run the simulation; now the late positive current plateau disappears. Print the results, and label your print. You should have a family of 3 curves which clearly identify the contributions of Na^+ and K^+ . Similar results can be obtained by running simulations where first external Na^+ is reduced to a minimal value (the lowest the computer will allow), then Na^+ is reset to normal and internal K^+ is reduced to a minimal value.

Results obtained in these and similar experiments over a range of values for E_{set} provided the basis of our current understanding of excitation and the voltage clamp technique remains one of the most powerful analytical tools in membrane biology. It allows you to study in detail how the membrane potential governs ion channels.

C- Strange Action potentials: Scorpion Toxin

The sodium channel agents discussed in the Experiment 1-J blocked the Na^+ conductance of the channel and reduced the action potential height. In this experiment we will look at the action of an alpha scorpion toxin that produces bizarre looking action potentials. First you will observe how the normal action potential changes as more and more scorpion toxin is applied. Then you will perform tests to elucidate where and how the toxin is acting.

Task

Load experiment Toxicology: Scorpion Toxin and set up the display. This experiment will be run in approximately the same way as the channel blocker experiment. In this case, however, the toxin is applied to the whole nerve, not to just a patch of the axon.

Using the slider, set the percentage inhibition to different values of your choice in the range from 0 to 100%. Click on the O (overlay) button and select several values (especially in the higher ranges > 75%) to show the dramatic changes in the action potential as more and more toxin is applied. Print your results.



To identify the site of action of the toxin, load experiment Voltage Clamp: Scorpion Toxin and set up the display. To check out the K^+ channels, poison the Na^+ channels, by selecting Membrane from the Set Parameters menu, and set $nNa = 0$. Run the simulation and overlay the responses for several levels of toxin (include 0% and 90%). Print your results. Now, to check Na channels, reset nNa to 1 and set $nK = 0$ so that all of the K^+ channels are poisoned. Again run the simulation and overlay the responses to several levels of toxin (including 0% and 90%). Finally, print these results. From the voltage clamp curves you should be able to distinguish the site of action of the toxin. Do you think the toxin exerts its affect on:

- a) the K^+ gates?
- b) the fast Na^+ gates?
- c) the slow Na^+ gates?

Why?

Do you think it makes these gates respond faster or slower?

Can you use your conclusions to interpret the shape of the action potential?

**Overview**

The voltage clamp experiments that you have run in exercises are simulations of an ideal feedback exerting perfect control over the membrane voltage. In this experiment we simulate a more realistic case where control is less than perfect because there are unavoidable delays caused by the time it takes the system hardware (voltage recorder, amplifier, current source, electrodes) to respond to any deviation in the membrane potential from its targeted value, E_{set} . As a result the “instructions” sent back to the current source, as well as the compensating current sent to the membrane always lag behind; they are always a little outdated. As you will see this causes errors; the actual membrane potential continuously deviates from the targeted E_{set} . Attempts to force it closer to the target may cause the system to become unstable.

Task

Open, and run the experiment. Compare the traces of E and E_{set} . In the previous experiments with the ideal voltage clamp they were identical but now E never seems to really match the targeted value E_{set} . Why? In the ideal experiment the identity between E and E_{set} was forced by a mathematical artifice - they were defined to be equal. To understand the more realistic simulated clamp, we need to follow the “chain of command” in more detail.

Interpret**Gain and Error**

When the Run button is clicked, the recorder/amplifier sends the appropriate amount of charge to the membrane to make the membrane potential equal to the desired E_{set} , and the membrane will respond (it has been stimulated). To maintain E close to E_{set} , the recorder/amplifier monitors E at all times and continuously “instructs” the current source to add positive or negative charge via the current source electrodes to stop E from drifting and to minimize the error. The actual “instruction” is simply an electrical signal proportional to the difference between E (the membrane potential) and E_{set} . This difference $E - E_{set}$ is defined as the **error**, and the proportionality constant is called the Gain. The compensating current sent to the membrane by the current source is then

$$I_{comp} = - \text{Gain} (E - E_{set}) = - \text{Gain} (\text{error})$$

The Gain is really the sensitivity of the control system - with a large Gain, a small error will induce a large feedback response. . The negative sign in front of the gain shows that whenever the error is positive (i.e. E is more positive than E_{set}), the current source compensates by sending negative charge to the membrane.

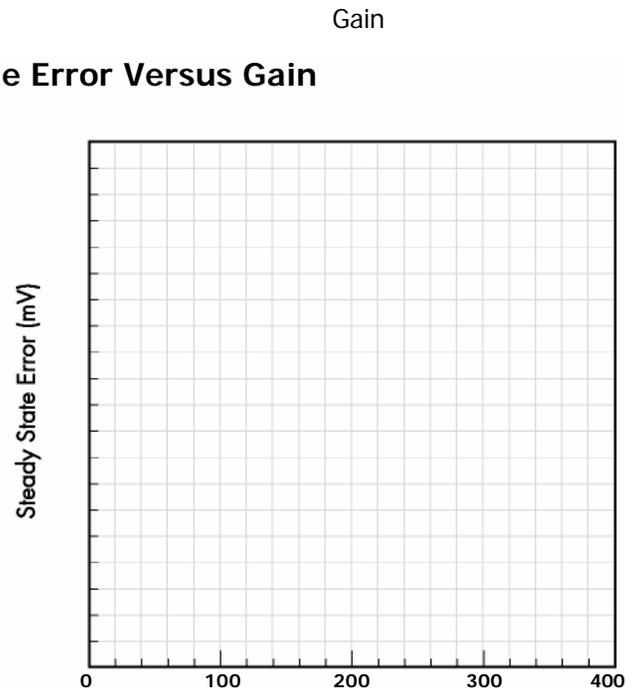


Task

Returning to the result of the first run, click on the I_{comp} button to remove it from the plot. E and E_{set} should be plotted on the display (if they are not , click on their buttons). The steady state error revealed by deviation of E from $E_{set} = -20$ mv. This is a common property of feedback systems. It occurs in natural physiological regulators like the endocrine system as well as in hardware that we have simulated. You might expect that the error could be reduced by increasing the sensitivity of the system. Can you reduce this by increasing the Gain? Can the error be reduced to zero by making the gain larger and larger? Increase the Gain until you find the transition point where the feedback becomes unstable. This instability shows up as wild oscillations in E or become too large for the computer to handle. (In the experimental hardware analog, the amplifier output voltage either oscillates or saturates) or as the Run 8 simulations with different values of Gain and plot the error (y axis) versus the Gain (x axis) below. Mark the maximum usable gain (minimum error) on your graph

Steady State Error Versus Gain

Gain	Steady State Error (mV)





Stability

As the gain gets larger the system becomes unstable because the outdated instructions are too large, causing I_{comp} to overcompensate. When this overcompensation is large E will overshoot its target by a wide margin. The control system then tries to correct this overshoot by sending a large signal in the opposite direction. As this hunting for the target continues the system may show sustained wild oscillations or the currents and voltages may grow larger and larger until the system breaks down (amplifier saturates).

Positive Feedback

A functioning voltage clamp is based on negative feedback. In our example $I_{comp} = -\text{Gain} (\text{error})$. Try changing it to a positive feedback. Using even a small gain = -1 so that $I_{comp} = \text{error}$ leads to an explosive instability.



Overview

Open Problem 1 and run the experiment. You will see two action potential recordings. In these simulations there are two axons under your electrodes, a normal control and an experimental which has had one of its properties altered. Action potentials from the normal axon (colored brown) are denoted by E , and those from the experimental (colored purple) are denoted by E_p . When you run the stimulator you excite both axons with the same stimulus pattern. Further if you change the external ion concentrations or the temperature, the same conditions will hold for both axons. Each time you run the experiment both action potentials are recorded. You will be able to change any of the properties of the control, but you will not be able to change properties of the experimental. :

Task

Your problem is to give a descriptive workup of the difference between these two axons. This should include a simple description of the action potentials that you see as well as how they both respond to any of the measurements that you have learned to make. Your decision on what to include and how to describe it is part of the problem.



Overview

Open Problem 1 and run the experiment. You will see two action potential recordings. In these simulations there are two axons under your electrodes, a normal control and an experimental which has had one of its properties altered. By properties we mean any of the following:

- internal Na concentration
- internal K concentration
- membrane capacitance
- number of voltage activated Na channels
- number of voltage activated K channels
- number of leak channels
- rate constant for opening fast Na channels
- rate constant for closing fast Na channels
- rate constant for opening slow Na channels
- rate constant for closing slow Na channels
- rate constant for opening K channels
- rate constant for closing K channels
- axon radius

Action potentials from the normal axon (colored brown) are denoted by E , and those from the experimental (colored purple) are denoted by E_p . When you run the stimulator you excite both axons with the same stimulus pattern. Further if you change the external ion concentrations or the temperature, the same conditions will hold for both axons. Each time you run the experiment both action potentials are recorded. You will be able to change any of the properties of the control, but you will not be able to change properties of the experimental.

Task

Your problem is :

1. Give a descriptive workup of the difference between these two axons. This could include a simple description of the action potentials that you see as well as how they both respond to any of the measurements that you have learned to make. Your decision on what to include is part of the problem.
2. Try to diagnose which property has been changed. You may attempt to mimic the experimental axon by altering the properties of the normal one. Once you



have decided which property has been altered, study the effects of changing the magnitude of the alteration until you arrive at a good estimate of how much the property has been perturbed in the experimental axon. Summarize the evidence you have gathered which supports your conclusions.

3. Give a qualitative account of why your diagnosis makes sense. This should take account of how the action of the channel gates leads to the observations you have cited.

4. Examine whether your interpretations are unique. Are there any other alterations that could have produced the same observations that you have cited?