

General instructions for Labs 1-4:

The lab reports in these labs will be in the form of Powerpoint slides. You will be instructed to incorporate screenshots or plots into Powerpoints slides. At the end of each lab session, you should leave a copy of your "unfinished" powerpoint file in the lab computer. This file will be your "raw data" notebook for the record. You should also copy the same file in a USB drive or your laptops. You need to finish figure legends or answers to questions in the manuals, in the "notes" section under the slides. Two out of the four members of your group will do a 5 minute presentation using your "finished" powerpoint file at the beginning of the next week's lab. You should also hand in your "finished" power point files. Grades will be determined by the quality of your presentation (50%) and your writing in the "finished" powerpoint file (50%). All members of the same group receive the same grade for both presentations and powerpoint files.

To prepare for the presentation, you should ask yourselves: (1) are the contents scientifically correct? (2) Are your statements/sentences clear and informative? (3) Are you facing the audience and talking to them?

Note that while we would like to see that you all provide correct answers in your presentation, mistake in answering questions are not fatal. However, we, Lab Instructors or Learning Assistants, would like to talk to you and clear up the mistakes you have made. The talk or discussion will be informal, a few minutes during or after the lab should be enough. The idea is not to leave issues or problems unaddressed.

These four labs will account for 25% of the total lab grades. The lab specific distributions will be: Labs 1 and 2: 5% each, Labs 3 and 4: 7.5% each.

Lab. 1

The purpose of this lab is to introduce you to the basic properties of action potentials. We will first familiarize ourselves with the user interface of the simulator. We will then perform current clamp experiments, to learn how action potentials (APs) behave under different experimental conditions. The experimental conditions we will explore include changes in: Na^+ concentration, g_K and g_{Na} .

1. Familiarize yourself with HHsim and action potential threshold.

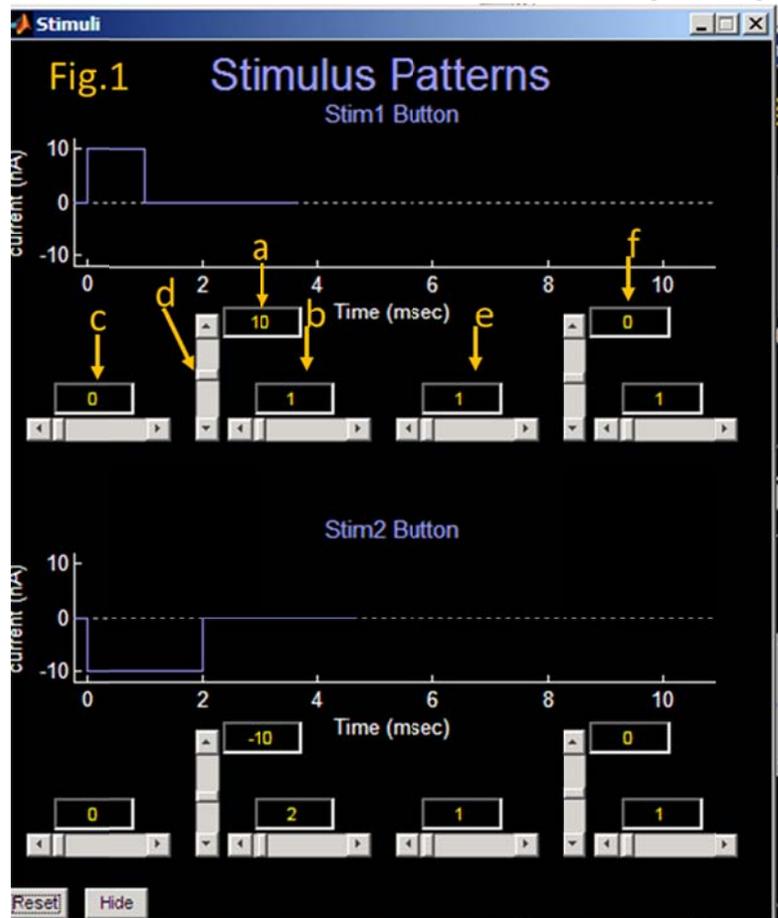
HHsim is a simple simulation program that allows you to explore membrane excitability. The first task is to learn the basics of this program in a few simple steps. We will start by learning how to stimulate a neuron.

HHsim should already be open on your computer. The first thing you need to do is to change two display settings. Near the bottom of the simulator window, there is a yellow window with a menu button to its right. Select g_{Na} (μS). In the green window to the right, select g_K (μS) and select "blank" in the cyan window.

Click on the stimuli tab at the upper left corner. The "Stimulus Patterns" window should appear (Fig.1). The default setting is to inject a 10nA current step (a) with 1 ms duration (b) at the beginning of a current trace (c).

You can change these variables—amplitude, duration and time of onset—by typing the required numbers in boxes next sliders, or by simply using the sliders (d). The blue line above all the boxes and sliders is the stimulation display in time scale. Have a good look at its X and Y-axes and make sure you see the step on the blue line and numbers in the boxes match.

To test the effect of this current step, go back to the simulator window, and click the "clear" button at the bottom of the window. Click it twice, so that traces in both top and bottom panels are cleared and reappear.



To test the current injection, click "Stim1", the blue button in the lower left corner. You should see an action potential (AP) in red in the top panel, while your current injection will be indicated by the blue trace below the red. In the lower panel, sodium and potassium conductance ,g_Na (yellow) and g_K (green), will be displayed.

Project 1: What is the current threshold for AP?

Go back to the "Stimulus Patterns" window and decrease the current amplitude in 1nA steps and write down the highest current that fails to fire AP. Starting with this largest subthreshold current, increase the value for current in 0.1nA steps until you find the lowest current that can fire AP. However, finish reading this page first.

Write down your current threshold in the note window of your powerpoint slide 1. (Don't just write the number, include unit as well, also make clear that the number is for Project 1 etc.) Paste the screenshot with AP initiated by your threshold current in the graph window of slide 1. (The best way to insert screen shot is to, in Powerpoint application, use Insert→Screenshot to port your HHsim window into powerpoint.) Save your file with the filename in the following format: Answers XXXX date where XXXX should be the initials, first and last, of every member in your group.

Also, in the note window, describe/compare g Na and g K in terms of their width, heights and onset. (By onset I mean which one takes off first?)

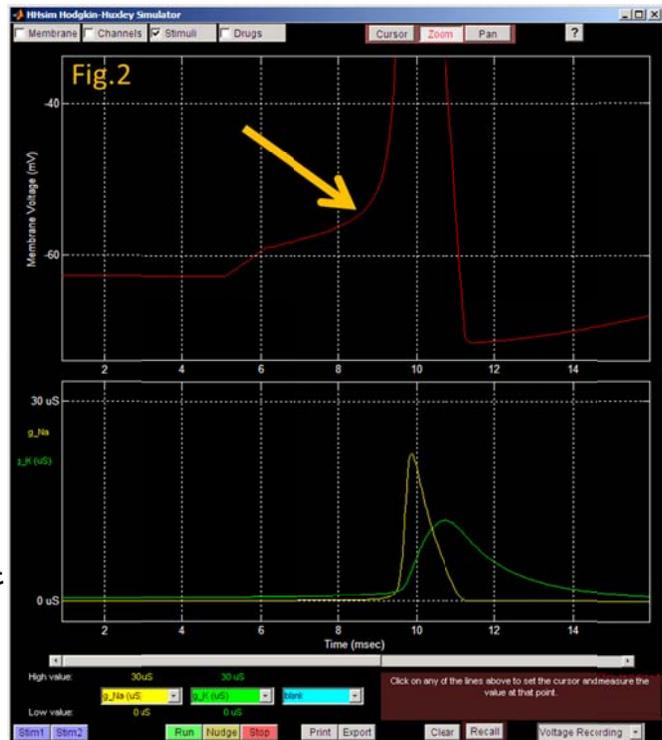
***This powerpoint file will be your raw data file and should not be changed further after you finish the lab today. You should take a copy of this file home, to polished it for the presentation next week. You will also need to complete figure legends and answers to some of the questions in this manual.

***Make sure that you click on the "Clear" button to clear the traces in the window every time you test a new current amplitude. The 1st click clears the top panel and the 2nd clears the bottom panel. It is a good idea to repeat this "double click" a few times (3 times-6 clicks) to be sure that the program goes back to the same numerical ground state before a new test is carried out.

***It should be noted that we are defining **current** threshold for firing an AP. In your textbook, when the threshold of AP is mentioned, it is often referring to **voltage** threshold, namely the voltage at which AP takes off with a sharp up-turn. In fact, the precise point when "AP takes off with a sharp up-turn" is not easy to define unambiguously. The area where AP starts to take off is enlarged in Fig.2. The arrow indicates roughly where the threshold is.

Project 2: What would be the current threshold of a 2nd current step if it were delivered 5ms after the first one?

We now need to add a 2nd current step. First, define the interval between the two steps by setting the number in the box labeled in Fig.1e to 5ms. Set the current amplitude of both the 1st and 2nd steps to 10nA (a&f). Click "Stim1". What happens?



You now need to find the current threshold for the 2nd step. Start by increasing the current of the 2nd step, in 10nA steps, until you see the second AP. Once you find the current that fires AP, decrease it in 1nA steps until you find the threshold. (Don't change the amplitude of the first current step, only the second one). What is the current threshold of the second step? Write down your answer in the note window of slide 2. Paste the screenshot with the 2nd AP initiated by threshold current in slide 2.

Compare the current threshold of the 2nd AP with that of the 1st AP—the one you determined from **Project 1**.

The experiment you have just performed is an example of the relative refractory period. The relative refractory period defines a time interval after an AP, during which a stronger stimulation is needed to fire an AP. Can you explain the mechanism behind this observation?

Hint: The reason why a stronger stimulus is required for the 2nd AP has to do with g_K and g_{Na} . Inspect the g_K traces in the lower window. Has g_K returned to baseline when the 2nd stimulus was delivered? (g_{Na} definitely has.) How would this "residual" g_K makes it harder to fire the 2nd AP? You don't need to answer this question immediately. However, you should have a 3-5 line answer in the powerpoint file you hand in next week. The powerpoint file you used for your presentation. (If you are not sure about the answers: talk among yourselves, ask Learning Assistants or Lab Instructor. Keep track of time though.

Perhaps it is better to save the question/discussion after you have finished all projects of this lab.)

The role of g_{Na} is not obvious from the g_{Na} trace. Why is the g_{Na} associated with the 2nd AP smaller? Come back and answer this question after you finish Project 7.

Project 3: Is AP amplitude dependent on extracellular Na^+ concentration?

We are now going to recreate a historically important experiment. This experiment provided the first conclusive evidence for the hypothesis that Na^+ influx was responsible for action potentials. We will use the same "Stim1" you played with in Project 1.

(1) Go to the "Stimulus Patterns" window. Click the "reset" button at the lower left corner.

(2) Click on the "Membrane" tab in the upper left corner of the simulator window, and you should see a "Membrane" window. Here you have the concentrations and Nernst equilibrium potentials for Na^+ , K^+ and Cl^- . Have a good look and make sure that you find the concentrations and E_{ion} .

(3) Go back to the main window and click on the "Stim1" button. You will get your AP as before.

(4) Along the top edge of the main window, there is a cluster of three buttons: Cursor, Zoom and Pan. Make sure that "Cursor" is highlighted in red.

You now need to measure the AP amplitude, by taking the difference between voltage at the peak of AP and at resting membrane potential (V_m). Click on the part of the red line where you think resting V_m should be. (Hint: It should be at the part of the red trace before the current step starts.) You should see an oval cursor on the red line, and the numerical readout indicated in the pink window at the lower right.

Click on the peak of the AP, and you will again find the voltage level of the AP peak in the pink readout window. The difference between AP peak and resting V_m should give you AP amplitude, or height. You will also need to enter Na^+ equilibrium potential (E_{Na}), found in the "Membrane" window.

You can now measure AP amplitude for the Na^+ concentrations (Na_{cn_out}) listed in the table below. You change Na^+ concentration by going to "Membrane" window and entering numbers in the box below C_{out} (mM). By the end of this project, you should have all the boxes filled, except perhaps the last row. Don't forget the double clicking "Clear" button routine.

Resting V_m (mV)	AP peak (mV)	AP amp (mV)	Na_{cn_out} (mM)	E_{Na} (mV)

			55	
			75	
			100	
			200	
			400	
			600	
			700	

It should be obvious why we are not going to record the reading for 700 mM Na⁺. This is not a software glitch in HHsim. When Na⁺ influx is very strong as a result of high extracellular Na⁺, there is enough residual Na⁺ current after each AP to push V_m beyond threshold and fire AP again. No need to go into detail here, but you should be aware that many neurons in our brain are capable of doing something like this, namely firing continuously in an autonomous way.

We now can use these measurements to make a nice graph, as good looking as that of the historical experiment. ([The effect of sodium ions on the electrical activity of giant axon of the squid.](#) HODGKIN AL, KATZ B.J Physiol. 1949 Mar 1;108(1):37-77)

Use "Alt_Tab" to find the "Lab 1" folder, and open the "Project 3 AP_Na_cn_plot.pxt". You should see a table with cells filled with 0, and a blank graph. Enter the relevant numbers from your measurements into the cells. As the numbers are entered, you should see the graph gradually taking shape. Look through the labels on the axes. The keys for the data, namely the red dots and grey line, are also indicated and the colors of the axes are matched with those of the data. The key point is that the AP_amp vs Na⁺ concentration plot has a slope similar to that of the Nernst equation, suggesting that Na⁺ permeability plays a key role in AP generation. For the IGOR file, use "Save As" to save your graph, with the file name in the format of "Project 3 AP Na cn XXXX", where XXXX should be the initials of everyone in your team.

Highlight the graph by clicking on it. Go to "EDIT" menu → Export Graphics. There will be an "Export Graphics" dialogue box. Click "OK". You can then paste the graph in the slide 3. Here, you need to write a figure legend. Figure legend should have an informative title: what is this figure meant to show. In the legend, you need to describe the graph. Although axes are labeled, you still need to say what have you manipulated and what have you measured. Although normally not done in scientific papers, we would like you to finish the legend with a 1-2 sentence conclusion about the meaning/significance of this graph. (Statements about the significance of a figure belong to text section of a paper, not figure legends. Also see Appendix for an example of figure legend.) As the answers associated with other slides, you don't need to have the legend in its finished form right now. However, it should be finished/polished when you hand in your powerpoint file next week.

Project 4: What happens to AP when you change g_K?

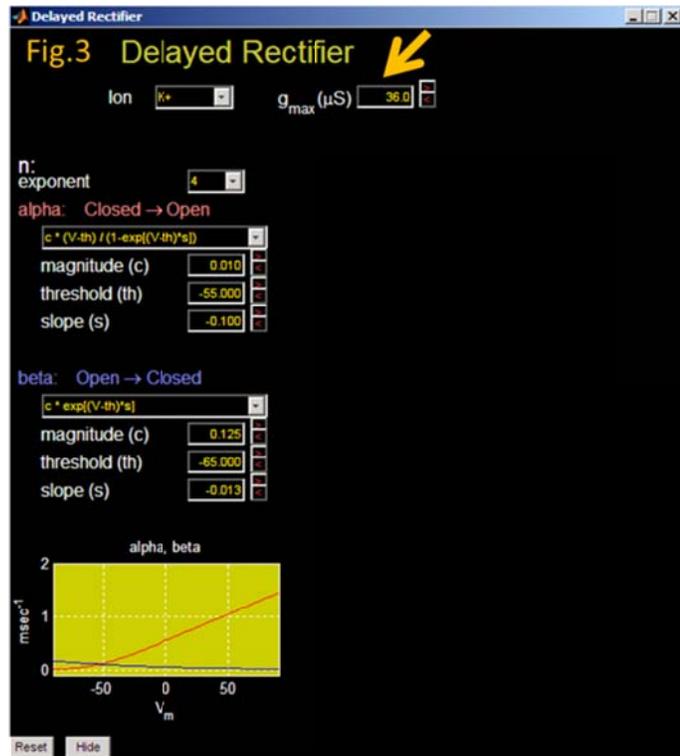
The purpose of this project is to gain some insights to the importance of K^+ channels. There are about 100 genes for K^+ channels. This large number gives rise to great variety of K^+ channels. K^+ channels are often the key to determining the shape, amplitude, firing frequency and firing pattern of AP. We will look at only one specific aspect of K^+ channel function here, namely how g_K values shape AP amplitude and duration.

(1) Go to the "Membrane" and "Stimulus Patterns" windows and click on the reset buttons.

(2) Make sure that the current step in Stim1 is 10nA.

(3) Click on the "Channels" tab near the top left corner, and the channels window should appear.

(4) Click on the "Details" button next to "Delayed Rectifier". (Delayed rectifier is the main K^+ channel in the squid giant axon. Naming a K^+ current Delayed Rectifier, a term originated from electric engineering, is a bit unfortunate. Hodgkin and Huxley built their own voltage clamp amplifiers, they must have been familiar with all concepts in electronics and electric engineering. This term is still commonly used today, to intimidate students with bio and psy background.) You should see the "Delayed Rectifier" window (Fig.3). It is a complicated window but we will change only one parameter, g_{max} , which is indicated in Fig.3 by the arrow. g_{max} represents the maximal conductance for the delayed rectifier and its value is proportional to the number of delayed rectifier K^+ channels available to this neuron—namely the larger the number, the more K^+ channels. You will test the effect of changing g_{max} , according to the values in the table below, on AP amp and duration. (The default g_{max} is $36\mu S$, reset button doesn't change this number.)



You know how to measure AP amp from "Project 3". What about measuring AP duration? It is somewhat arbitrary. We will use the AP width at -40 mV as its duration. To be able to place the cursor precisely, you will need to zoom in a bit. Click on the Zoom tab at the top of the simulator window. The cursor will turn into a little magnifying glass. Zoom in on the AP you want to measure at the level of -40 mV, then go back to the top of the window and click on the cursor tab in order to measure the times at which the red, AP lines

cross -40 mV line. (Zooming in by 2 clicks should be enough. If you zoom in the wrong place, just right click, zoom out and try again.) You will now be able to measure the times at which the **rising** and **falling** phases of the AP cross the -40 mV line, by clicking on the red trace and placing the oval indicator there. The time corresponding to the pink oval is displayed in the black window to the right of the pink window you used to read voltage. (At this level of zoom, the oval cursor may not fall exactly on the point at which AP crosses the -40mV line. Don't get stressed, just get the cursor as close to the -40mV line as possible.) Write down the times at which the AP line crosses the -40 mV line. The difference between the two time points is the duration. Write down your duration in the table.

g_{\max} (μ S)	AP peak (mV)	AP amp (mV)	AP duration (ms)
33			
36			
40			
50			
60			
70			

Zoom out and measure AP amplitude. (Don't forget to take the difference between baseline and the peak to obtain AP amplitude.)

Bring up the "Lab 1" folder and open the "Project_4 g_K variation plot.pxt" file. You can now enter the numbers you have just measured. Save the IGOR file and paste the graph in powerpoint slide 4 and write a figure legend for it.

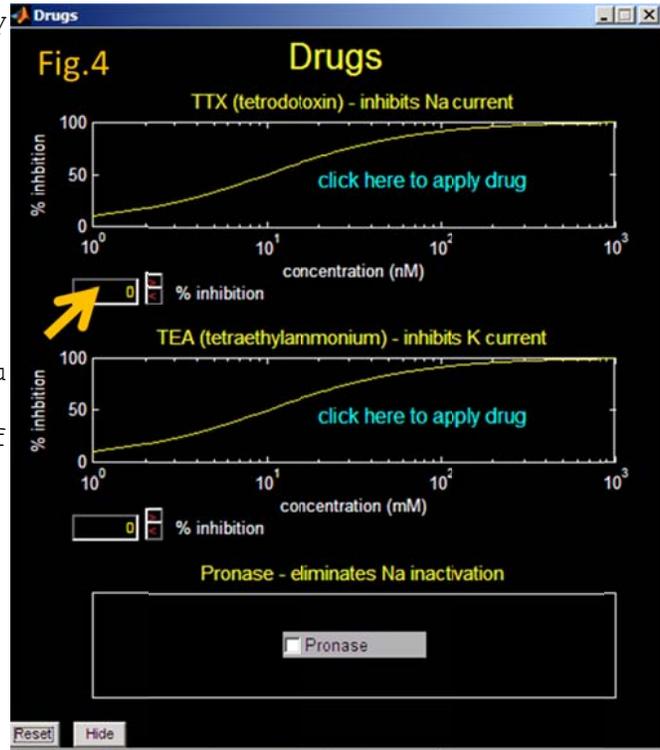
Pay attention to the trend in AP duration and amplitude as the g_K value is increased. In general, higher g_K level results in lower AP amplitude and narrower AP duration. Explain why this is so in your presentation next week. Don't forget to add your explanation in the powerpoint file.

Project 5: What happens to AP when you change g_{Na} ?

There is another way to change voltage gated conductance, namely using channel blockers.

(1) Reset the "Membrane", "Channels" and "Stimulus Patterns" windows.

(2) Click on the "Drug" tab in the simulator window. You should see a window labeled "Drugs" (Fig.4). We are going to ignore the cyan writing that invites you to click on the graph. (Click on Reset at the lower left corner if you have already clicked on the graph.) For the purpose of graphing, it is more convenient to define the level of blocking numerically. We will enter the percentage of Na^+ channel block by hand (Fig.4 arrow). Let's start by increasing the level of block in 5% increments. Measure AP amp as before, you will also need to measure g_{Na} by placing the cursor on g_{Na} trace, lower panel.



% inhibition	AP amp (mV)	g_{Na} (μS)
0		
5		
10		
25		
30		
40		
50		

Bring up the "Lab 1" folder and open the "Project 5 g_{Na} block plot.pxt" file. You can now enter the numbers you have just measured. Don't forget to save your plot and paste the graph in slide 5, write a figure legend. Explain, in your own words, why the reduction in g_{Na} results in reduced AP amplitude. Explain in your presentation next week and in the note window of slide 5.

It is somewhat surprising that AP can still be generated after 50% of the channels are blocked. This is not unrealistic. Most neurons in our brain have a lot more Na^+ channels than is necessary to fire AP, generally by a factor of 2-10. In neurophysiology terms, we say neurons have very high safety factor for generating AP.

Lab. 2

In Lab. 1, we explored AP behavior by inspecting how AP amplitude and duration changed as we manipulated $[Na^+]_{out}$, g_{Na} and g_K . Experiments this week are designed to investigate mechanisms underlying AP in greater depth. We will start by using voltage clamp experiments to examine i_{Na} and i_K activated by voltage steps. We will then consider the directions of current flow by incorporating the core concept of Nernst equilibration with voltage clamp experiments. We will finally consider the behavior and significance of Na^+ channel inactivation.

Project 6: How to you analyze and present Na^+ and K^+ current recorded with voltage clamp?

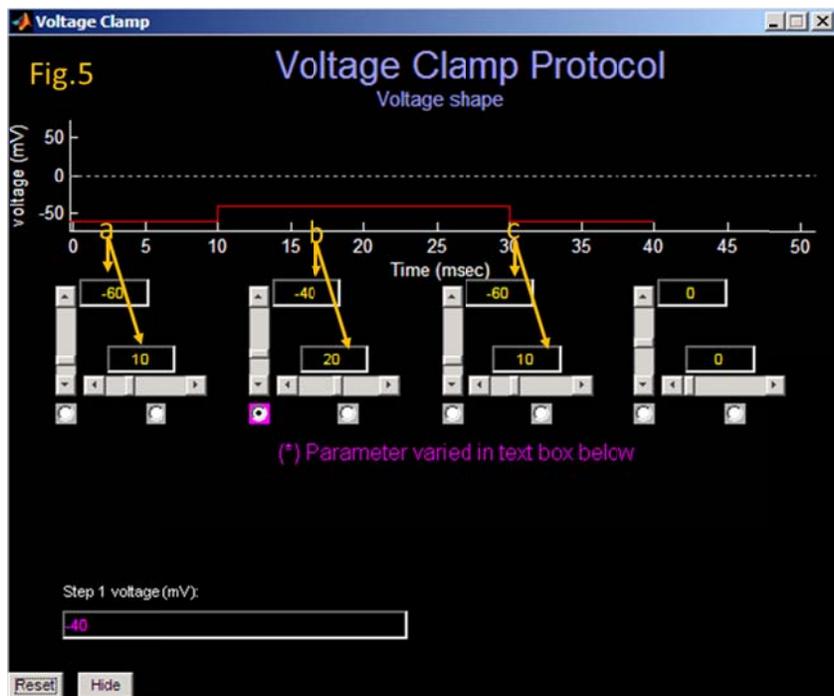
(1) HHsim allows us either to inject current into an axon and record voltage responses (current clamp) or to perform voltage clamp and measure current. We can switch between these two modes with the tab at the lower right corner of the simulator window. Switch to Voltage Clamp now.

As a reminder, all projects in Lab. 1 were current clamp experiments where we manipulated current injection into an axon and observed membrane potential (V_m) and AP changes. In lab. 2, we will use both current clamp and voltage clamp techniques. Voltage clamp is a technique where we control V_m of a neuron and measure current flow through ion channels, while the voltage is maintained at a constant level.

Once you turn on Voltage Clamp, the "Stimulus Patterns" window will change into "Voltage Clamp Protocol", Fig.5. The red line in this window defines how we will control membrane potential: starts at -60mV for 10ms (a) then step to -40mV for 20ms (b), then step back to -60mV for 10ms (c).

These voltage levels are not ideal. We are going to change the **holding potential to -90 mV by changing the numbers in (a) and (c) to -90mV**. For the

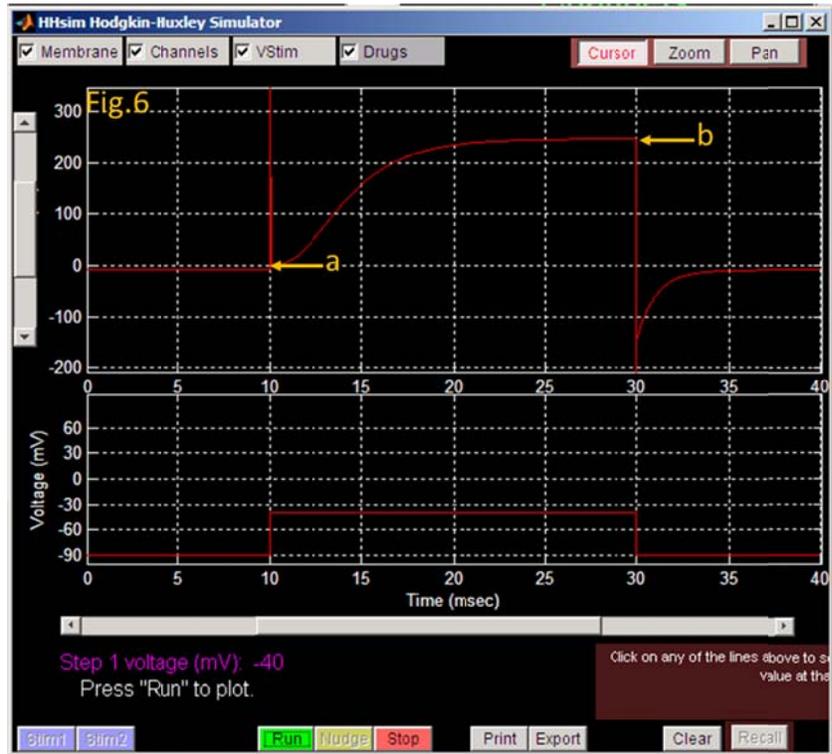
purpose of illustration, our voltage step will be -40mV (b). There is no need to change time.



(2) We want to keep things simple and analyze one current at a time. Go to the "Channels" window and **unchecked** "Fast sodium" so that we can analyze i_K in isolation.

(3) Click "Run" in the simulator window. (A reminder, use "Run" when you simulate voltage clamp but use "Stim1" when you perform current clamp, by injecting current and record V_m responses.)

The current in the upper window will look a bit odd. Don't worry about the details. We will focus on how to measure this current. In theory, we should use an approach similar to that for the measurement of AP amplitude, namely by taking the difference between the baseline (Fig.6a) and the maximal i_K amplitude (b). In the case of i_K , the maximal current is always at the end of the voltage step. However, since the baseline (a) is very near to zero, taking the reading from (b) alone, without subtraction, should be good enough. Again, you should click on the part of the trace you want to measure, and take the reading from the pink box at the lower right. (Pay attention to the units in the current readout.) You need to run the voltage steps shown in the table below, by going back to the "Voltage Clamp Protocol" window and changing the number in the voltage step box (Fig.5b). (Don't change anything in the lower, time box.) Also note that the voltage you enter should be the absolute voltage, i.e. the voltage level where the steps jump to, not the height of the voltage step.



Voltage (mV)	i_K (nA)	i_{Na} (nA)
-80		
-60		
-40		
-20		
0		
20		
40		

(4) After you finish all the voltage steps and measure the corresponding i_K . Go back to the "Channels" window. **Check "Fast**

Sodium" and uncheck "Delayed Rectifier". Use the same voltage steps to activate i_{Na} and fill in the column of i_{Na} in the table.

Note the signs on the current reading. Also note that i_{Na} measurement should be taken from the minimal point of the current.

Switch to the "Lab 2" folder and open the "Project 6 I-V plot" file. You know the routine by now. Enter the numbers in the appropriate cells in the table, and you will get your beautiful I-V plot. Save your plot and export your plot to slide 1. Write a figure legend for this slide.

Compare your plot with Fig.3.3 in your textbook. Your i_K plot should be similar to the "late" line in Fig.3.3, while your i_{Na} line should be similar to the "Early" line. Hopefully, this exercise will have given you a better idea about what an I-V plot is. Construction of an I-V plot is a way to summarize the behavior of i_{Na} and i_K as the V_m is changed by voltage clamp. Since this is a survey course, we will not torture you about detailed reasons why the I-V curves of i_{Na} and i_K look so different. However, it is educational to think about parameters that determine the direction of current flow with one small slice of the voltage clamp experiment.

Project 7: What does the concept of Nernst equilibration have to do with the direction of i_{Na} flow?

In the I-V plot you constructed for Project 6, i_{Na} was inward and negative going while i_K was outward and positive. The question we will address here is: what factors determine the directions of Na^+ or K^+ flow? The answer hinges on the understanding of Nernst equilibrium. The voltage clamp experiment in this project is designed to help us think through this problem.

Here, we are going to use one voltage step, -60mV to 0mV, to open Na^+ channels. However, the voltage step will be tested in three $[Na^+]_{out}$: 440, 50 and 10 mM.

(1) Click "Reset" of the "voltage Clamp Protocol" window then change the voltage step from -60mV to 0mV (the upper box of Fig.5b).

(2) **"Channels" window: make sure that "Fast Sodium" is checked and "Delay Rectifier" is unchecked.**

(3) "Membrane" window: click "Reset"

(4) "Simulator" window: Click "Clear" a few time. Click "Run". i_{Na} activated by the voltage step show appear.

(5) "Membrane" window: change $Na^+_{C_{out}}$ to 50mM. Head over to "Simulator" window: **Don't click the clear window this time. Just click "Run".** Blue i_{Na} and voltage traces should appear. (We want to overlay traces so we can compare them.)

(6) "Membrane" window: change $\text{Na}^+_{\text{C}_{\text{out}}}$ to 10mM. Head over to "Simulator" window: **Don't click the clear window this time. Just click "Run".** Green i_{Na} and voltage traces should appear.

Inset the screenshot in slide 2. and write figure legend.

Of the three i_{Na} traces, the red is negative, blue is flat and green positive. Explain this observation in the note section of slide 2, following the figure legend.

Hint: Think about what we have changed, $\text{Na}^+_{\text{C}_{\text{out}}}$.

What happen to E_{Na} as $\text{Na}^+_{\text{C}_{\text{out}}}$ was changed?

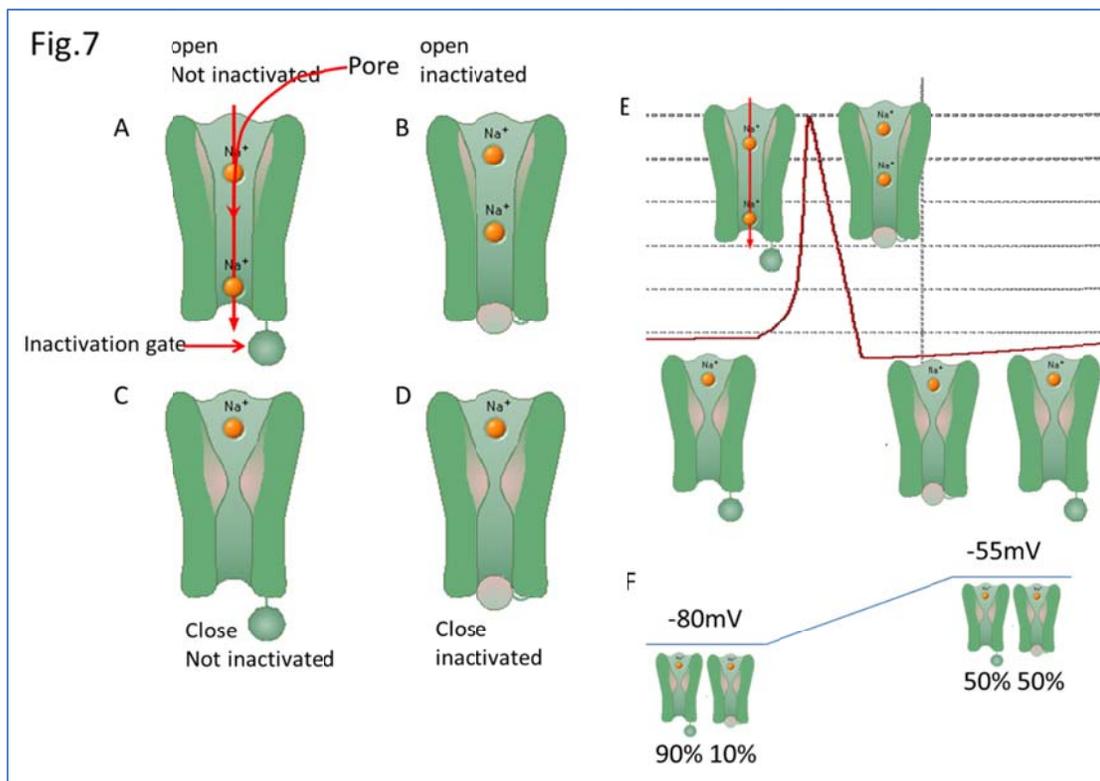
What happen to Na^+ channel conductance change, due the voltage step to 0mV, as $\text{Na}^+_{\text{C}_{\text{out}}}$ was changed? (Note that Na^+ channel conductance is mainly determined by depolarization and that we use the same voltage step for all of the three $\text{Na}^+_{\text{C}_{\text{out}}}$ values.)

What kind of force, electric or diffusional, is driving Na^+ when Na^+ channels open and Na^+ is allowed to pass through the channel pore?

Try to think through these questions for each Na^+ concentration.

Project 8: How does Na⁺ channel inactivation modify the ease/difficulty in AP initiation?

By now, we all have the expectation that when V_m is depolarized, Na⁺ and K⁺ channels will open. However, Na⁺ channels have another layer of complexity, namely inactivation process. Figure 7A-D illustrates the four states a Na⁺ channel can have. At a negative V_m , most channels are in close and not_inactivated state (C&E). The rising phase of an AP is caused by inward Na⁺ current after the channel pore open (A&E). However, even for a brief event such as an AP (~1 ms in duration), a significant fraction of Na⁺ channels become inactivated and stop carrying Na⁺ influx on the falling phase (Fig.7B&E). After the AP is over and V_m return to resting level, the channel pore will return to closed state (Fig.7D&E) and then the inactivation gate will disengage (Fig.7C&E). This inactivation process is extensively exploited by mother nature and can be found in many variants of Na⁺, K⁺ and Ca⁺⁺ channels.

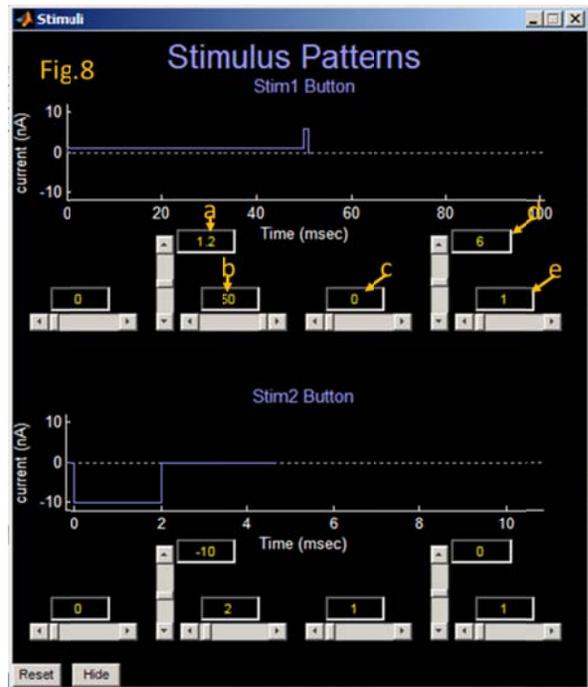


More interestingly, Na⁺ channels can also "sneak" into inactivated state from closed state if resting V_m of a neuron is slightly depolarized for a long time (Fig.7F). For example, if the resting V_m of a neuron stays at -80mV, 90% of its channels will be in closed and not_inactivated stage (Fig.7F). (The remaining 10% will be in closed and inactivated state (Fig.7F).) Under this condition, 90% of the Na⁺ channels will open and carry current when the neuron is stimulated. In contrast, if the same neuron shifts its resting V_m to -55 mV, only 50% of its Na⁺ channels will be available—namely in closed

and not_inactivated state—while the remaining channels are not available because they are inactivated already (Fig.7F). (Note that those "closed and inactivated" channels cannot contribute to AP because their channels pores are "plugged" by the inactivation gate even though depolarization could open the pore.) Thus, it would be more difficult to fire AP when this neuron has a more depolarized V_m . In fact, many neurons in our brain can subtly adjust their resting V_m and shift the fraction of Na^+ , K^+ or even Ca^{2+} channels between closed-not_inactivated and close-inactivated states. You can then imagine that depending on the type of inactivating channels a neuron has, depolarizing resting V_m could reduce excitability if Na^+ channels is the main channel type inactivated by depolarized V_m .

(1) Switch back to "Voltage Recording" in the simulation window. Reset parameters in the following windows and then close them: "Drugs", "Membrane" and "Channels". (Make sure that both "Fast Sodium" and "Delayed Rectifier" are checked. Reset the "Stimulus Patterns" window.

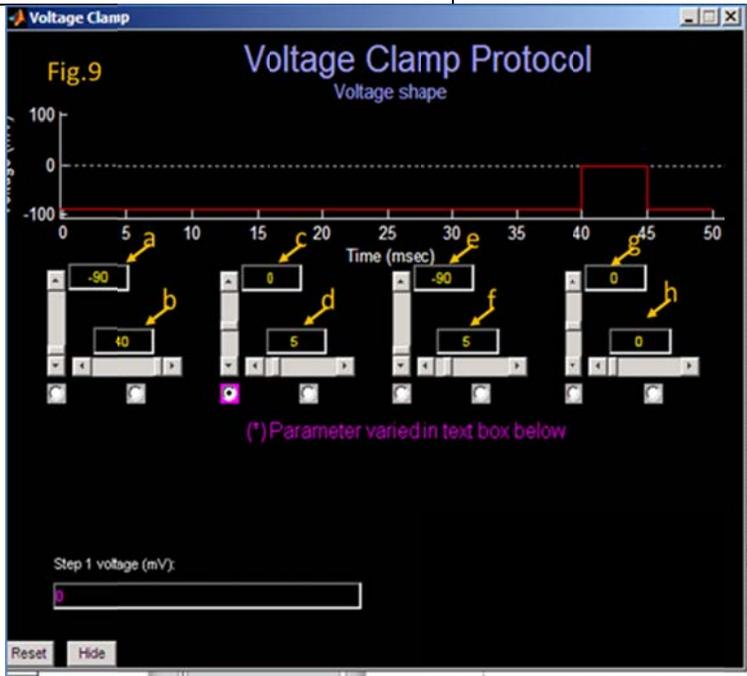
(2) We are going to change resting V_m for 50ms and see how this change in V_m alters AP amplitude. Refer to Fig.8 to set up this protocol. Set the conditioning current to 1.2nA (a) and the duration to 50ms (b). Set the next time window to 0ms (c). Finally, set the 2nd current step to 6nA (d) and 1ms (e). The 50ms period before the stimulus (d and e) current step is called the conditioning step. Use the current steps listed in the table below to see how these conditioning current steps affect resting V_m and AP amplitude. (Don't forget to do the following: Near the bottom of the simulator window, there is a yellow window with a menu button to its right. Select g_{Na} (μS). In the green window to the right, select g_{K} (μS) and select "blank" in the cyan window.)



Note: resting V_m should be measured right before the 2nd, 6nA, current is delivered, at ~50ms point. We want to measure V_m that has been modified/conditioned by the 50ms conditioning current steps. (Note that the conditioning current here is used to modify resting V_m for 50ms. Although only 50ms, it is already enough to significantly alter AP amplitude because it is long enough to engage inactivation process and shift some channels from the closed-not_inactivated state to the closed_inactivated state (Fig.7F). Many neurotransmitters in our brain can modulate resting V_m over the time scale of minute to hours. During this period, neuronal excitability will be altered because the

balance between closed-not_inactivated state and closed_inactivated state of channels will be shifted.)

Conditioning current (nA)	Resting Vm (mV)	AP amplitude (mV)
1.2		
1		
0		
-1		
-2		
-5		
-10		



Don't forget the routine of clicking on the clear buttons etc. Open "Project 8 Inactivation.pxt" to plot these measurements (Table 0: I_{cond} , AP_{amp}). Leave the plotting file open, since there is a 2nd part to this project.

The second part of this project is to observe inactivation with voltage clamp (switch the mode from "Voltage Recording" to "Voltage Clamp"). Since we are going to look at i_{Na} alone, open the "Channels" window, and unclick "Delayed Rectifier" to turn i_K off.

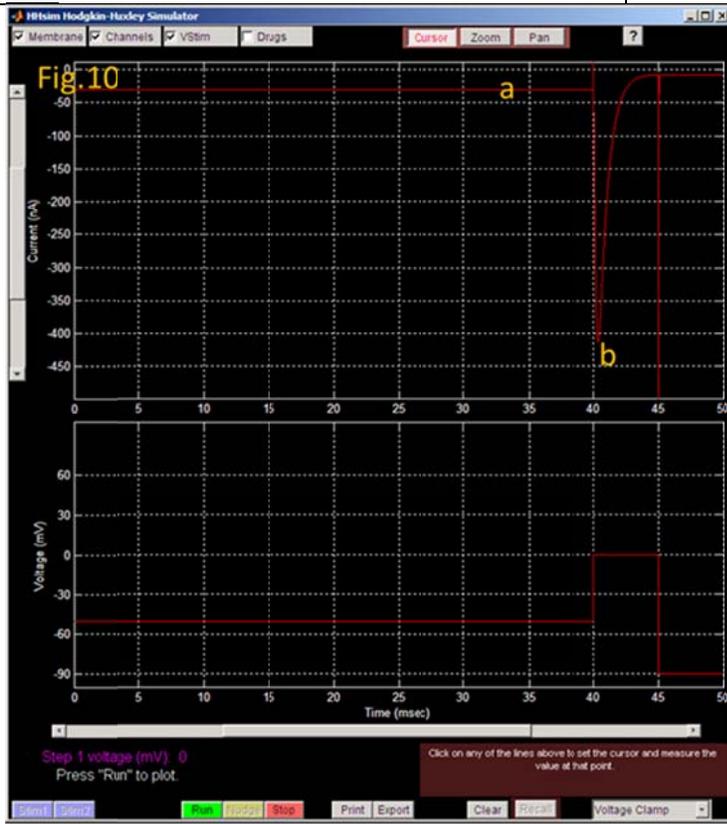
Set the parameters in the "Voltage Clamp Protocol" window exactly as indicated in Fig.9. The main parameter we are going to change systematically is the value in "a". This value sets the level of voltage for the 40ms (b) conditioning pulse. We will then measure i_{Na} amplitude activated by a 5ms depolarizing step to 0mV (Fig.9 c and d). Also set numbers in boxes e, f, g and h as indicated in Fig.9.

Note: Since we are interested in how the conditioning pulses at different V_m may affect i_{Na} by an inactivation process, we need to

monitor i_{Na} using a constant voltage step, namely the 5ms step to 0mV defined by c and d in Fig.9.

Also note that we need to measure i_{Na} amplitude by taking the difference between baseline and peak i_{Na} , namely between a and b in Fig.10 because baseline i_{Na} may not be zero.

Conditioning voltage (mV)	i_{Na} (nA)
-90	
-80	
-60	
-50	
-40	
-35	



Type in the values Table1: V cond, I Na. Export both plots to slide 3. Write a figure legend for each.

What is the significance of inactivation?

Look at the i_{Na} vs conditioning voltage plot and consider the fact that a normal neuron has a resting membrane potential range of between -50 to -70mV: such a neuron can dial its available Na^+ channels down to <20%, at -50mV, or dial upward to ~70% of maximal i_{Na} at -70 mV. In other words, as this neuron shifts its resting V_m , which in turn regulates the percentage of Na^+ channels inactivated, its ability to fire AP (called "excitability") can be finely controlled by regulating

the fraction of Na^+ channels available for opening and generating AP. (Not to belabor the point, but Na^+ channels that are not inactivated are the ones that can carry current and generate AP.)

***Don't forget to go back to the Answers file and answer the last part of Project 2 on the role of inactivation in relative refractory period.

Consider and discuss these questions:

1. Are there more Na^+ channels in closed-inactivated state when the second stimulus was delivered than the moment right before the first stimulus? Why?

2. Can you then explain the reason why the threshold for firing the second AP is higher than that of the first AP on the basis of Na^+ channel inactivation?

3. If we wait for a long time before delivering the second stimulus, say 500 ms instead of 5 ms as we did in Project 2, there threshold for firing the second AP will be the same as that of the first AP. Can you explain this observation?

Appendix:

This figure and legend below illustrate how you should write figure legend. You describe experimental conditions underlying each trace. As a convention, that is all you need to do, namely describe the figure.

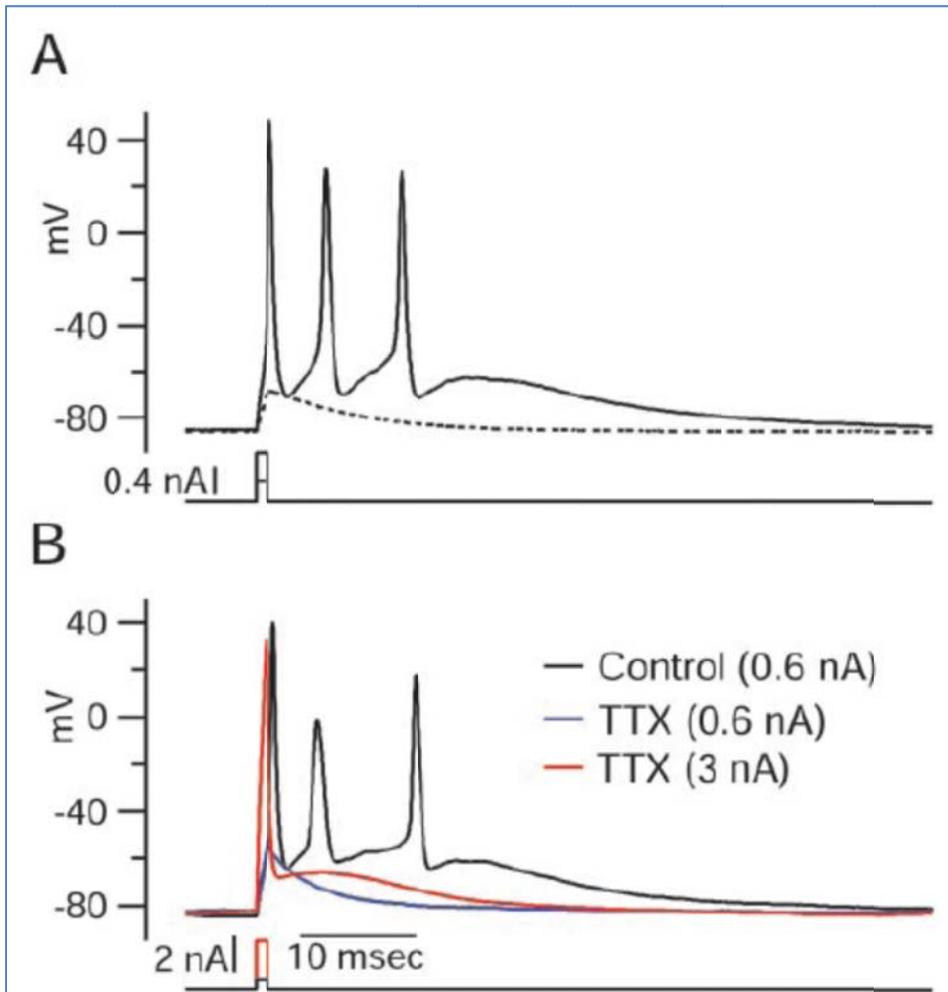


Figure 1. Burst firing in dissociated Purkinje neurons. *A*, All-or-none burst elicited in response to a brief (1 msec) depolarizing current injection. Dashed trace shows passive response to current injection to just below the voltage threshold. *B*, Effect of $2 \mu\text{M}$ TTX. TTX completely blocked the active response elicited by a moderate suprathreshold current injection (blue), but with a very large (3 nA) current injection for 1 msec (red), the response during the stimulus (passive response to +30 mV, followed by repolarization caused by activation of potassium conductance) was followed by an afterdepolarization.