

# Sample HHSim Exercises

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## Part I: Equilibrium Potential

We will first explore the equilibrium potential of a cell with a single channel type. Click on the Channels button in the main window to call up the Channels window. In the Channels window, turn off all the channels *except* the first one (passive sodium), by clicking on their respective buttons. The buttons should change color to gray when you turn them off. Notice that the resting potential  $V_r$  displayed in the Membrane window is now equal to the reversal potential for sodium, 52.4 mV. A mouse click on the red line will show you the exact value of  $V_m$ .  $V_m$  may not be exactly equal to  $V_r$ , but clicking the yellow **Nudge** button a few times will nudge things along until the voltage reaches the theoretical asymptote. Or you can click **Run**, wait a while, and then click **Stop**.

- Question 1. Calculate the effect of halving the external sodium concentration. Remember, we're assuming that only sodium channels are present. Use the simulator to confirm your answer by looking at the value of passive  $V_r$  in the Membrane window. (You can type new values into any of the boxes in the Membrane window, or use the < or > buttons to change the values.)
- Question 2. Calculate the  $[Na^+]$  external concentration required to achieve  $V_r = 55.5$  mV. Confirm your answer using the simulator.

Hit the **Reset** button in the Membrane window to restore all parameters to their initial values.

- Question 3. Suppose we double the temperature from 6.3° C to 12.6° C. What is the new value of  $V_r$ ? Explain why  $V_r$  doesn't double.

## Part II: Membrane Potential

In exercise we will look at the membrane potential with only passive channels (no voltage gated sodium or potassium channels.) Reset the simulator by typing "run" again in the Matlab Command Window. (Don't hit the green **Run** button; make sure the simulator is stopped before typing "run" in the Command Window.)

Turn off the yellow and green plots in the main window by selecting "-" in their respective pull-down menus. Set the cyan (light blue) plot to "I\_leak", the leakage current. I\_leak is equal to the flow of current through the passive channels. By looking above and below the cyan pull-down menu, you will see that the scale of the I\_leak plot ranges from -0.05 to 0.05 pico-Amp. Display the Membrane and

Channels windows as well.

In the Channels window, turn off the two voltage gated channels (pink buttons) so that only the three passive channels (green buttons) are functional. Note that the membrane voltage (red line) rises from its previous level of roughly -62 mV to a new level of around -48 mV. The simulator stops when the membrane voltage is close to asymptote. To get an exact resting value, click the yellow **Nudge** button a few times until the value displayed for  $V_m$  stops changing.

The rise in  $V_m$  shows that at the normal resting potential, some voltage-gated channels as well as leak channels were open. When the voltage-gated channels are disabled, the only open channels are the leak channels, and resting membrane voltage rises. Since membrane current must be 0 at  $V_{rest}$ , the leak current (blue line) is zero.

- Question 1. Write down the parallel conductance equation for the resting potential as a function of equilibrium potentials and conductances. Given the equilibrium potentials shown in the Membrane window and the conductances shown in the Channels window, calculate the resting potential  $V_r$ .
- Question 2. You can see from the Channels window that  $g_K$  is 0.070 micro-Siemens. Calculate to two significant digits the value to which  $g_K$  would have to be reduced in order to make  $V_r$  be -45 mV. Verify your answer by changing the value of  $g_K$  in the simulator and seeing how the value of passive  $V_r$  in the Membrane window changes.
- Question 3. We can also manipulate the resting potential  $V_r$  by changing the ion concentrations. Obviously it is easier to change the concentrations outside the cell than inside. Suppose we want to raise the cell's resting potential to -39 mV, while maintaining a constant osmolarity and not disturbing the charge balance. What ion concentrations should we change, and what should their new values be? Use the simulator to find the answer empirically.

## Part III: The Action Potential

Reset the simulator by typing "run" again in the Matlab Command Window. Click on the purple **Stim1** button in the main window to stimulate the cell and generate a spike. You can see the stimulus plotted as a purplish line, while the spike is visible as the membrane voltage plotted as a red line. Click on the **Stim2** button to apply a hyperpolarizing pulse to the cell. Notice that this also results in a spike.

- Question 1. Why does hyperpolarization cause a spike?

Click on the Stimuli button in the main window to call up the Stimulus Parameters window. Notice that button **Stim1** is set up to provide a 1 millisecond stimulus at +10 nano-amperes. Using the slider controls, you can add a second pulse to **Stim1**. Set up a second 10 nA pulse 5 msec after the first. (Note: clicking on the slider changes the value by 5. Clicking on the arrowheads changes the value by 1. You can also type values directly into the box next to each slider. Moving the mouse over a slider or text box displays some pop-up text explaining its function.)

Notice that two pulses spaced 5 msec apart do not cause two spikes.

- Question 2. Why doesn't this second pulse cause a second spike? Phrase your answer in terms of gates and voltages.

- Question 3. How much time must elapse between the end of the first pulse and the beginning of the second in order for the second pulse to cause a second spike? (Use the simulator to experiment.)

A negative pulse occurring soon after a positive pulse can prevent a spike from occurring. Set up the **Stim1** parameters to provide a 5 nA pulse followed 1 msec later by a -5 nA pulse. You'll see that the 1 ms interval is too long to prevent the spike. Shorter intervals can be obtained by typing a fractional value like 0.9 into the text for the middle horizontal slider.

- Question 4. To the nearest 10th of a millisecond, what is the longest delay after a 1 msec 5 nA positive pulse that a 1 msec -5 nA pulse can block a spike?

## Part IV: The Fast Sodium Channel

Type "run" in the Matlab Command Window to reinitialize the simulator. Press the **Stim1** button to stimulate the cell and generate a spike. The yellow, green, and cyan (light blue) lines in the upper plot display the Hodgkin-Huxley variables  $m$ ,  $h$ , and  $n$ , respectively. The variables take on values between 0 and 1. Recall that the fast sodium channel conductance is proportional to  $m^3h$ , and potassium channel activity is proportional to  $n^4$ .

In this exercise we will focus on just the sodium channel, which consists of an activation gate and an inactivation gate. Both these gates must be open in order for current to flow. The  $m^3$  term describes the state of the activation gate, and the  $h$  term describes the state of the inactivation gate. The closer these values are to 1, the more "open" the gate is.

Using the cyan pop-up menu, set the cyan plot to display  $g_{Na}$ , the sodium conductance, which is proportional to  $m^3h$ . The numbers above and below the yellow, green, and cyan windows represent the y-axis values of the uppermost and lowest dashed lines, respectively, in the upper plot. The range for the cyan plot is now 0 to 30 pico-Siemens. Click on **Zoom In** to get a better view, and use the scroll bar to position the plot as necessary.

As you can see, the inactivation gate variable  $h$  (green line) has a resting value of around 0.5, so it's normally part-way open. But the activation gate variable  $m$  (yellow line) normally rests close to 0. This is why the total sodium conductance (cyan line) is close to zero pico-Siemens at rest.

When a stimulus depolarizes the cell slightly, the activation gate opens (yellow line " $m$ " rises), the sodium conductance (cyan line) increases, and the cell depolarizes further (red line  $V_m$  rises). However, as the membrane voltage increases, the inactivation gate begins to close (green line " $h$ " decreases to zero), and this causes the overall conductance (cyan line, proportional to  $m^3h$ ) to drop even though the yellow line remains high (activation gate remains open) for quite a while.

What eventually causes the yellow line to drop is the repolarization of membrane potential (indicated by the red line dropping) as a result of opening of voltage dependent potassium channels. Without the potassium channels, the cell would remain depolarized. To demonstrate this, we will do an experiment.

Call up the Channels window and disable the Delayed Rectifier (potassium) channel. There is normally some leakage current through this channel when the cell is at rest; removing it triggers a spike. The cell

depolarizes but cannot repolarize, because now there is no active potassium current. The membrane voltage thus remains high, and the yellow line stays high.

- Question 1. looking at the simulator output, at what potential does the cell settle after the spike has subsided, i.e., what is the new resting value of  $V_m$ ? Click on the red line to measure the value.

The spike peak is around +50 mV. With no active potassium channel conductance, the cell does not fully repolarize, but the membrane voltage does decline some from the peak value. Notice that if  $V_m$  is perturbed from its new resting value by a depolarizing or hyperpolarizing stimulus (try the **Stim1** and **Stim2** buttons) it returns to the new resting value.

The new value of  $V_m$  is determined by the conductances of two channel types: the leak channels (which have conductance to Na, K, and Cl), and the (not quite fully closed) fast voltage dependent sodium channels. Although it may appear that in this state  $h = 0$  and hence  $g_{Na} = 0$ , they are actually slightly positive. Because the leakage conductance is so low, the slight steady-state activation of voltage gated Na channels still has a large effect on  $V_{rest}$ .

- Question 2. Using the parallel conductance equation, calculate the conductance of the fast sodium channel in this new resting condition.
- Question 3. Stimulating the cell again (using the **Stim1** button) in this condition will not cause another spike. Even if you raise the stimulus intensity to 20 nA and the duration to 10 msec, the cell will not spike. (Try it. Turn off the cyan plot by selecting "-" in the pop-up menu so you can see the yellow and green plots clearly when you hit **Stim1**.) What is the explanation for this?

## Part V: The Delayed Rectifier

Type "run" in the Matlab Command Window to reset the simulator. Press **Stim1** to stimulate the cell and generate a "normal" spike, for reference. Then call up the Channels window, and disable the fast sodium channel. The resting potential hyperpolarizes only slightly, indicating that very few voltage dependent Na channels are open under normal resting conditions.

Turn off the yellow plot (select "-") in the pop-up menu and set the green plot to "I\_K", the delayed rectifier current.

Call up the Stimulus window and set Stim1 to a 100 nA stimulus lasting 5 msec. Press the **Stim1** button in the main simulator window to depolarize the cell, and see what happens.

- Question 1. Based on the  $V_m$  plot (red line), what is the peak value reached by the membrane voltage? Click on the line to the right of the peak, and use the < button to find the maximum.
- Question 2: after reaching its peak, the membrane voltage quickly declines again, even though the stimulus is still on. What is causing this? Point to some evidence to support your answer.
- Question 3: when the stimulus ends, the cell does not simply return to its resting value; it undershoots it and then approaches the value from below. Why doesn't it just return to its resting value?

## Part VI: Voltage-Gated Channel Parameters

Hodgkin and Huxley explained the behavior of voltage-gated channels in terms of activation and inactivation gates that opened or closed based on the membrane voltage. These "gates" are pieces of channel structure (i.e., segments of the amino acid chains that make up the channel) which change their shape or position based on the potential across the membrane. The  $m^3$  term in the expression  $m^3h$  for the fast sodium channel indicates that three segments of the channel must change their conformation to open the activation gate, while the  $h$  term indicates that only a single piece of structure controls the inactivation gate.

At the molecular level channel components do not just sit in a fixed position at temperatures above absolute zero,. They continually change their conformation. However, some conformations are more likely than others, and therefore occur more frequently. The channel characteristics determine which configurations are more likely, as a function of the current membrane voltage. The parameter  $m$  can be viewed as the probability that a particular segment of the activation gate is in the configuration required for the gate to be open. Alternatively, it can be viewed as the fraction of channels in which this segment is in the configuration required for the gate to open. Since all three segments must be in the open position for the activation gate to be open, and the segments are independent, the probability that the gate is open (or equivalently, the fraction of channels with open activation gates) is  $m^3$ .

Type "run" in the Matlab command window to reset the simulator. Call up the Channels window, and press the Details button for the fast sodium channel. This displays a window showing the parameters responsible for the behavior of the fast sodium channel. Looking at the top of the display, we see that this channel is permeable to the sodium ion, and that its maximum conductance  $g_{\max}$  is 120 micro-Siemens.

- Question 1: how does  $g_{\max}$  for the fast sodium channel compare with the passive sodium conductance?

The left half of the Fast Sodium details window describes the activation gate, while the right half describes the inactivation gate. The behavior of an activation gate segment is governed by two rates:  $\alpha$  is the rate at which segments move from the closed to the open state (causing  $m$  to increase), while  $\beta$  is the rate at which they move from the open to the closed state (causing  $m$  to decrease).  $\alpha$  and  $\beta$  are determined by exponential functions whose parameters are shown in the window. These equations are functions of  $V_m$ . In the graph at the bottom,  $\alpha$  is plotted in red, while  $\beta$  is plotted in blue.

Looking at this plot, we see that when  $V_m = -50$ ,  $\alpha$  is much less than  $\beta$ . That means segments will move from the open to the closed configuration at a higher rate than they move from closed to open. As a result, the net change in  $m$  will be a decrease.

- Question 2: What relationship must hold between the red and blue lines for  $m$  to increase? And, what is the approximate value of  $V_m$  at which this condition occurs (if there were no other mechanisms affecting the cell)? How does this compare to the cell's normal resting potential?

Now press **Stim1** to trigger a spike, which we will use for reference.

The state of the inactivation gate is indicated by the variable  $h$ , whose parameters are displayed in the

right half of the Details window. Suppose we want to lengthen the duration of a spike. We can do that by slowing down the rate at which the inactivation gate closes, so that the channel remains open longer. Beta is the rate at which the inactivation gate will close. Change the beta magnitude parameter ( $c$ ) from 1.0 to 0.4. Then press the yellow **Nudge** button in the main simulator window a few times. The cell starts spiking and doesn't stop. Press the red **Stop** button to stop the simulation. You should notice several things:

1. The spike width is now greater, as we intended.
2. The green line ( $h$ ) decreases more slowly during a spike, and does not get as close to zero as it did previously. This is the direct consequence of our decreasing the  $h$  beta magnitude.
3. The yellow line ( $m$ ) stays high for longer. This is because the cell is remaining depolarized longer. Remember that it is the closing of the inactivation gate ( $h$ ) that cuts off the sodium influx and allows the cell to repolarize. If the gate closes more slowly,  $V_m$  stays high longer, and hence the activation gate remains open longer.
4. The peak of the spike is higher than before.

As you can see, the cell is quite sensitive to parameter changes; altering a single value produces a complex set of effects. We may conclude that the channels governing a cell's behavior form an exquisitely tuned system. And here we're only dealing with two channel types; keep in mind that some cells have a dozen!

- Question 3: in order to stop the cell from oscillating on its own, we can change the passive channel conductance. Hit the **Run** button in the main simulator window to continue the simulation. By playing with the value of  $g_K$  in the Channels window, find a value close to the original value of 0.07 micro-Siemens that prevents the cell from spiking spontaneously. The cell should of course still spike in response to a stimulus from the **Stim1** button. Report the  $g_K$  value you find, to two significant digits.

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