

Exercise 2: Hodgkin and Huxley model

Expected time: 4.5h

To complete this exercise you will need access to MATLAB version 6 or higher (V5.3 also seems to work), and the Hodgkin-Huxley simulator code. At the Matlab Command Window you need to go to the directory containing the simulator code. Type "run" in the Matlab Command window and the simulator will start. The main simulator window should pop up on your display. The simulator will run for a few milliseconds and then stop. In the bottom left corner of the main simulator window are three buttons labeled "Membrane", "Channels", and "Stimuli". Click on the "Membrane" and "Channels" buttons to make those windows visible on the display. Move them to convenient positions.

A. Equilibrium Potential

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 colour 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. Note that V_m displayed in red in the main window when the simulator stops may not be exactly equal to this value, 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 (points: 2): *Calculate the effect of halving the external sodium concentration. Remember, we're assuming that only passive 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.) Show the calculation and the results.*

Question 2 (points: 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 (points: 2): *Suppose we double the temperature from 6.3°C to 12.6°C. What is the new value of V_r ? Please explain shortly, why V_r doesn't double.*

B. Membrane Potential

In this part of the 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 "blank" in their respective pulldown 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 V_r , 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_r , the leak current (cyan line) is zero.

Question 1 (points: 1): *Write down the parallel conductance equation for the resting potential V_r as a function of equilibrium potentials E and conductances g . 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 (points: 2): *You can see from the Channels window that the K^+ conductance 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 $V_r = -45\text{mV}$. 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 (points: 1): *Set g_K back to 0.070 micro-Siemens. 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 -39mV , while maintainning a constant osmolarity and not disturbing the charge balance. Explain shortly the term osmolarity in context of the resting potential manipulation. What ion concentrations should be changed, and what should their new values be? Use the simulator to find the answer empirically.*

C. 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 (points: 2): *Why does hyperpolarization cause a spike? Hint: Observe the variables m , n and h .*

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-Amps. Using the slider controls, you can add a second pulse to "Stim1". Set up a second 10 nA pulse 1 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 1 msec apart do not cause two spikes.

Question 2 (points: 2): *Why doesn't this second pulse cause a second spike? Phrase your answer in terms of gates and voltages. Consider the plot (yellow, green, and cyan lines) of m , h and n below the voltage. Recall that the fast sodium channel conductance is proportional to m^3h , and potassium channel activity is proportional to n^4 .*

Question 3 (points: 1): *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 (points: 1): *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? Note: in order to get accurate results, be sure that V_m has returned to its asymptotic value of -62.7 mV before supplying the next stimulus. Click on the "Nudge" or "Run" buttons to give the simulator time to reach asymptote.*

D. The Fast Sodium Channel

Press the "Stim1" button to stimulate the cell and generate a spike. The yellow, green, and cyan 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 g_{Na} is proportional to m^3h , and potassium channel conductance g_K is proportional to n^4 . In this part 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.

Turn off the yellow and green plots. 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.

Switch on the green plot to see the inactivation gate variable h (green line). It has a resting value of around 0.5, so it's normally part-way open. Switch on the yellow plot to display 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 g_{Na} (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 (points: 1): *Looking at the simulator output, at approximately what potential does the cell settle after the spike has subsided, i.e., what is the new resting value of V_m ?*

The spike peak is around +50 mV. With no active potassium channel conductance g_K , 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 S2 buttons) it returns to the new resting value.

The new value of V_m is determined by the conductances of two channel types: the passive leak channels (which have conductance to Na, K, and Cl), and the active (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_r .

Question 2 (points: 3): *Starting with the equation,*

$$c_m \frac{dV(t)}{dt} = -i_m$$

calculate the conductance g_{Na} of the fast sodium channel in this new resting condition.

Question 3 (points: 1): *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 "blank" 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?*

E. The Delayed Rectifier (active potassium channel)

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 "blank") 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 (points: 1): *Based on the V_m plot (red line), what is the approximate peak value reached by the membrane voltage?*

Question 2 (points: 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 (points: 2): *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?*

F. Voltage-Gated Channel Parameters

Hodkin 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 (points: 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 (points: 2): *What relationship must hold between the red (alpha) and blue (beta) lines for m to increase? Please describe first the common case and later the case for $m = 0.5$. In this case we assume, that no other effects affecting the cell. For $m = 0.5$, what is the approximate value of V_m and 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 and "Stim1". The cell starts spiking and doesn't stop. Press the red "Stop" button to stop the simulation. You should notice several things:

- The spike width is now greater, as we intended.
- 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.
- 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.
- 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 (points: 1): *In order to stop the cell from oscillating on its own, we can change the passive channel conductance, for example g_K . 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.*