Please make sure to do the reading prior to completing the problem set.

Action Potential I Lab Notebook

Reading: Action Potential I

Name:
Date:
Purpose of Lab:

Question 1: What is the effect of the hyperpolarizing current on the membrane potential?

Set the Total Duration of the simulation to 100 ms, set Number of Pulses to 1, set the Pulse Duration to 50 ms, set the Fast transient sodium conductance to 0, and the Delayed rectifier potassium conductance to 0 (the simulation will complain, but this is the easiest way to set the values, and it will still work). Inject hyperpolarizing current using the Stimulation Current First Pulse. Try currents of different magnitudes: -1 nA, -2 nA, -4 nA, -8 nA and -16 nA.

Please explain effect of the hyperpolarizing current on the membrane potential in terms of the passive properties of the nerve cell, i.e., the membrane capacitance and the membrane resistance (or conductance) that has not been set to zero. You may find it helpful to refer to the previous unit in the course to answer this question.

Now restore the Fast transient sodium conductance to 120 µS, and the Delayed rectifier potassium conductance to 36 µS. Repeat the experiments in part A with the same current values. *What do you observe? How do the active conductances that you have just restored to the membrane affect the responses you saw in part A of the question?* As you apply the different currents, carefully note the scale of the *y*axis. An action potential is defined as a change in voltage that goes from negative (resting) values to positive values (usually around +30 mV). Make sure to use this criterion in this and subsequent questions to determine whether or not what you are seeing is an action potential.

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Insert a screenshot of the simulation and label the figure.

Question 2: How do the responses to depolarizing currents differ from those to hyperpolarizing currents that you used in the previous question?

Using the same parameters as in Question 1B, inject depolarizing currents of different magnitudes (values to try: +1 nA, +2 nA, +4 nA, +8 nA, + 16 nA). *Explain your results.*

Insert a screenshot of the simulation and label the figure.

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Question 3: Find the minimum positive current that induces an action potential.

Again using the same parameters as in Question 1B, *find the minimum positive current that induces the cell to fire an action potential*. Note that you are using a 50 ms pulse in this problem, and the result will differ if the pulse length is different (for example, 4 ms, which is the default pulse length). *How sharp is this threshold value?* To address this question, obtain an estimate to 4 significant figures (you should not do this for the rest of the problem set; two or three significant figures will generally be enough).

Use binary search to narrow down the values.

- Let us say that 4 nA is too little to fire an action potential, and 8 nA is more than enough.
- Try the value midway between the two, i.e., 6 nA.
- Assume that 6 nA is not enough to fire an action potential. This narrows the interval of interest to the range from 6 nA to 8 nA. Choose the middle of the range, i.e., 7 nA.
- Assume that 7 nA is enough to fire an action potential. So now the range has been narrowed from 6 nA to 7 nA. Once again, choose the middle of the range, i.e., 6.5 nA.
- Binary search is very fast, because with each repetition, you halve the range, and this can allow you to narrow down to the correct answer within a relatively small number of steps. In general, this is a useful approach whenever you need to estimate parameters, which is likely to happen many times during the this course.

Pulse Duration:	50ms
Min Positive Current:	

Insert a screenshot of the simulation and label the figure.

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Question 4: What is the absolute refractory period?

To do this question, it is helpful to have a shorter pulse duration. Press the Reset button to return the pulse width to 4 ms, which is better for doing this problem. Make sure to set the number of pulses to 1, and set the simulation duration to 100 ms. The drawback is that you will now need to *find a new (higher) value for the threshold current*, since the pulse is shorter. Please do so using binary search; do not worry about 4 digits of accuracy.

Pulse Duration:	4ms
Min Positive Current:	

Now, set the number of pulses to 2 and the Inter-stimulus interval to 40 ms. Set the Stimulus current subsequent pulses to 70 nA, which is about the maximum amount of current one could inject into a real neuron without causing damage. Run the simulation. *What do you observe?*

Now, systematically decrease the Inter-stimulus interval by decrements of 5 ms, and re-run the simulation. *What is the shortest interval at which you can still evoke a second action potential?* Once you have found the correct general area, you may vary around it in 1 ms increments or decrements to get a somewhat more precise answer. This defines the **absolute** refractory period, i.e., the time before which a neuron *cannot* generate a new action potential in response to extremely strong (but not damaging) inputs.

Insert a screenshot of the simulation and label the figure.

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Question 5: What underlying mechanisms of the action potential could account for the **absolute** refractory period?

- Understanding the behavior of the ion channel gates before, during, and after the action potential is important for answering this question properly. Individual ion channels and their gates can only ever be in an open state or a closed state; the opening and closing of gates is a random process, and the probability of a voltage-dependent gate being in the open state is determined by the membrane potential.
- Neurons have many copies of each ion channel, so we can speak of populations of channels and gates. Since individual gates can only be open or closed and the process of switching states is random, in a large population of channels some gates of one type will be open and others will be closed.
- Notice the plot of the **m**, **h**, and **n** It shows you the proportions of each type of gate in the population that are open as a function of time. The **m** gates are represented by a blue line, the **h** gates by a purple line, and the **n** gates by a red line.
- Note also the sodium conductance and current are represented by solid blue lines and the potassium conductance and current are represented by solid red lines.

Once you have found the absolute refractory period in the previous question, set the Inter-stimulus interval so that the simulation can just generate a second action potential, and observe what happens to the **m**, **h** and **n** gates and the conductances and currents. Run it again with the Inter-stimulus interval shortened by 1 ms so that an action potential is not generated, again observing the **m**, **h** and **n** gates and the conductance and currents. *Explain how the gates change on either side of the absolute refractory period, and their effect on conductance and current.*

Question 6: Determine the relative refractory period, i.e. find a duration after the first action potential for which a pulse **of the same exact amplitude and duration** as the initial pulse can again evoke an action potential.

Change the Stimulus current subsequent pulses to the threshold current you found in question 4A (this should be the same value that the Stimulus current first pulse is currently set to). Start the Inter-stimulus interval at 40 ms for the second pulse, and shorten it by decrements of 1 ms. *When does the second action potential fail*? This time represents the end of the **relative** refractory period, i.e., after this time the neuron acts as if it has no memory of firing a previous action potential. *What underlying mechanisms of the action potential could account for the **relative** refractory period?* Once again, looking at the gates carefully will help you answer this question. *Explain in terms of the gates.*

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Insert a screenshot of the simulation and label the figure.

Look closely at the n and h gates shortly after the neuron has finished its after-hyperpolarization. *Are they in steady state? What would you predict will happen if you inject a second pulse equal or even slightly less than the threshold pulse during this time?* Run the simulation, observe what happens, and *explain in terms of the gates*.

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Insert a screenshot of the simulation and label the figure.

Question 7: What is the relationship of the amount of current injected and the number of action potentials that fire?

Press the Reset button. Set the Total Duration of the simulation to 400 ms, set the Pulse Duration to 390 ms, and set the Number of Pulses to 1. We can use this simulation to explore repetitive firing. Set the stimulus current for the first pulse so that you induce a single action potential. Once again, you may need to use binary search to find this value. Find the minimum amplitude of the pulse that induces two action potentials. Now, find the minimum amplitude of the pulse that induces three action potentials. Repeat this to find the current that induces four and five action potentials. *Plot the number of action potentials (on the y-axis) against the current needed to induce that number of action potentials (on the x-axis). How does the amount of current increase? Can this neuron easily use the number of pulses it generates to represent a linearly increasing input (e.g., a linear increase in mechanical deformation of the skin)?*

Number of Pulses	Minimum Amplitude
1	
2	
3	
4	
5	

Insert a plot and label the figure.

Question 8: Find the minimum current value that induces firing of action potentials throughout the pulse.

Measure the time between the pulses (use action potentials after the 5th to do this, to ensure that the value has stabilized; zooming in on the plot will make it easier to take these measurements). Call this period t. Convert the period into a frequency (in Hertz, Hz) by calculating 1000/t, since t is measured in milliseconds. Inject double, triple and quadruple the original current into the neuron, and redo the frequency measurement for each of these current levels. *Plot the amount of current injected (x-axis) against the firing frequency (y-axis). Based on your results, what kind of messages can a neuron like this send from one point to another? Can it faithfully represent a signal that changes linearly in intensity using its rate of firing? Explain.*

Current I	Period t	Frequency f

Insert a plot and label the figure.

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Question 9: Increase the current until the neuron no longer fires action potentials beyond the first. *Explain what might cause the failure of action potentials in terms of the gates.*

Insert a screenshot of the simulation and label the figure.

Discussion:

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