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

Action Potential IV Lab Notebook

Reading: Action Potential IV

Name:
Date:
Purpose of Lab:

Sag Current

Question 1: What does the reversal potential imply about the kinds of ions that permeate the sag current channel?

If you click on the simulation, you will see that it lists multiple potassium currents, sodium currents, a nonspecific current, and multiple calcium currents. Because looking at all these currents at once is confusing, the next few questions will allow you to examine them one at a time. The first conductance that we will examine is known as the sag or IH conductance (in the simulation, it is listed under the Nonspecific Currents as H-current). The reason for the name will become clear as we look at its effects. Note that the IH conductance's reversal potential is -38.8 mV. *What does this imply about the ions that permeate this channel? Could it only permeate potassium ions, or only sodium ions? Explain.*

Question 2: Explain the voltage change when injecting hyperpolarizing current.

Please click on the button labeled "Sag current". Please set the Stimulus current first pulse to -1 nA, the Stimulus delay to 50 ms the Pulse duration to 200 ms, and the Total duration to 300 ms. Under Potassium Currents, set the Delayed rectifier potassium conductance to 0 and under Sodium Currents, set the Fast transient sodium conductance to 0. Under Nonspecific Currents, set the H-current conductance to 0, and run the simulation.

Take a screenshot of the Membrane Potential, and Stimulation Current plots. Note the final voltage reached during the hyperpolarizing pulse, and also note its shape. *What features of the membrane are responsible for this voltage change?* Recall the electrical equivalent circuit equation that you worked with in the Passive Membranes unit.

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Insert a screenshot of the simulation and label the figure.

Question 3: How is the voltage response different when changing the H conductance?

Now set the H-current conductance back to 0.005 μS. Note the scale of the y-axis. *How does the voltage change during the initial part of the hyperpolarizing pulse? What is the maximum negative value that the voltage reaches after the pulse has been on for 200 ms?* ***Take a screenshot of the Membrane Potential, and Stimulation Current plots (2 plots).***

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The change in conductance and current due to the IH current is plotted in gray in the Sag Current, Conductance and Gate plots. *What is the IH current doing? Explain*, including the reason it is also known as the "sag" current. *What has happened to the value of the resting potential after the current pulse stops? Explain* in terms of the electrical equivalent circuit equation that you worked with in the Passive Membranes unit (Equation 7 in that unit). ***Take a screenshot of the Sag Current, Conductance, and Gates (3 plots).***

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Question 4: With the HH current restored and the sag current turned off, apply a long duration hyperpolarizing current followed immediately by a small depolarizing current. What happens?

Press the Sag current button again to restore the Hodgkin Huxley currents. To see what happens after a hyperpolarizing current pulse when no sag current is present, set the H-current conductance to zero, set the Stimulus current first pulse to -0.5 nA, the Stimulus current subsequent pulse to 0.1 nA, the Pulse Duration to 100 ms, the Inter-stimulus interval to 0, the Number of pulses to 2, and the Total duration to 250 ms.

Run the simulation, and observe what happens immediately after the hyperpolarizing pulse ends. *Please explain what you observe.* ***Take a screenshot of the Membrane Potential and Stimulation Currents (2 plots), and the Sag Current, Conductance, and Gates (3 plots).***

Insert a screenshot of the simulation and label the figure.

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Now re-set the H-current conductance to 0.005 μS. *What do you observe? Please explain* this in terms of the sag current and its effect on the Hodgkin Huxley gates. ***Take a screenshot of the Membrane Potential and Stimulation Currents (2 plots), and the Sag Current, Conductance, and Gates (3 plots).***

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Now set the Stimulus current first pulse to 0 nA, and again run the simulation. *What do you observe? What is the importance of the initial hyperpolarization for the action of the sag current?* ***Take a screenshot of the Membrane Potential and Stimulation Currents (2 plots), and the Sag Current, Conductance, and Gates (3 plots).***

Insert a screenshot of the simulation and label the figure.

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Calcium Conductances

Question 5: Observe the response of the membrane after the three calcium channels are added.

Nerve cells contain a wide variety of voltage-gated channels that allow the ion calcium to enter the nerve cell. Calcium is tightly regulated within nerve cells. The free ionic calcium concentration is usually kept at very low levels inside the cell relative to the extracellular concentration of free calcium using a variety of pumps, buffers, and special organelles that take up and sequester calcium (e.g., the mitochondria and the endoplasmic reticulum). In the next part of this problem set, we will explore three calcium conductances (T, N and P), their effects on excitability and their effects on intracellular calcium levels.

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To see how the neuron behaves in the absence of any of the calcium conductances, please press the button Simple simulation, *and observe the response of the model neuron before and after the current pulse.* ***Take a screenshot of the Membrane Potential and Stimulation Currents (2 plots).***

Insert a screenshot of the simulation and label the figure.

Now, please press the Calcium currents button, and *observe the response of the model neuron before and after the current pulse.* Looking at the current plots under the Calcium Currents, Conductances, and Gates, *which of the three calcium ion channels generates the largest current?* ***Take a screenshot of the Membrane Potential and Stimulation Currents (2 plots), and the Calcium Currents, Conductances, and Gates (3 plots).***

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Set that channel's conductance to zero, and again run the simulation. *What do you observe? Explain.* ***Take a screenshot of the Membrane Potential and Stimulation Currents (2 plots), and the Calcium Currents, Conductances, and Gates (3 plots).***

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Which of the two remaining calcium ion channels now generates the largest current? Set its conductance to zero, and again run the simulation. *What do you observe? Explain.* ***Take a screenshot of the Membrane Potential and Stimulation Currents (2 plots), and the Calcium Currents, Conductances, and Gates (3 plots).***

Insert a screenshot of the simulation and label the figure.

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Intracellular Calcium Concentration and Buffering

Question 6: Calcium accumulation and buffering

We can now examine the effect of the calcium currents on the accumulation of calcium within the nerve cell. To understand this properly, think about a buffer as a kind of "sponge" that soaks up calcium so that it is no longer in the neuron's cytoplasm. If the buffer has a short time constant, it works quickly to clear calcium from the cytoplasm.

Please press the Calcium currents button. Now set the T-current conductance, the N-current conductance, and the P-current conductance to zero. Run the simulation. *Please measure the amount of calcium at the beginning of the simulation and after 40 ms* (i.e. measure values from the Intracellular Calcium Concentration graph). In addition, ***take a screenshot of the Intracellular Calcium Concentration graph (1 plot)***.

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Insert a screenshot of the simulation and label the figure.

Please press the Calcium currents button again, which will restore the conductances of the calcium currents, and again measure the amount of calcium at the beginning of the simulation and after 40 ms. *What do you observe? Explain.* ***Take a screenshot of the Intracellular Calcium Concentration plot (1 plot)***.

Insert a screenshot of the simulation and label the figure.

How does the buffer affect the long term internal calcium levels? Understanding this will be very important for understanding the next conductance, the calcium-dependent potassium conductance. To explore this, change the value of the Calcium buffering time constant from from 25 ms to 5 ms. *Please measure the total amount of calcium that accumulates by the end of 40 ms. What do you observe? Explain.* Now change the Calcium buffering time constant to 100 ms. *Again, please measure the total amount of calcium that accumulates by the end of 40 ms. What do you observe? Explain.* ***Take screenshots of the Intracellular Calcium Concentration plots for these two scenarios. (1 plot each).***

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Calcium-Dependent Potassium Current

Question 7: Run the simulation with the calcium currents and the calcium-dependent potassium current. Contrast the result to question 5.

Calcium is not only an ion that permeates channels; it can act as an effector and second messenger within cells. Thus, fluxes of calcium not only contribute to changes in the potential of the membrane, but can also contribute to many intracellular processes. An example of one of its effects is its ability to affect a special potassium conductance. These channels open in response to both voltage and calcium levels. We can now use the simulation to explore the properties of this conductance.

Please press the Ca and Ca-dependent K currents button. Please change the Total duration of the simulation to 100 ms, and run the simulation. *What do you observe after the current pulse? How does the response of the neuron differ from what you observed in Question 5, when there was no calcium-dependent potassium current, but all three calcium currents were present? Please measure the membrane potential at 50 ms.* ***Take a screenshot of the Membrane Potential and Stimulation Currents (2 plots).***

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Insert a screenshot of the simulation and label the figure.

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Now examine the Calcium-Dependent Potassium Current, Conductance, and Gate. ***Take a screenshot of the Calcium-Dependent Potassium Current, Conductance, and Gate (3 plots).*** *Please use their changes to account for what has happened to the membrane potential.* To test your hypothesis, turn off the calcium currents (i.e., set their conductances to zero), and again run the simulation. *Measure the membrane potential at 50 ms. How has it changed? Explain.* ***Take a screenshot of the Membrane Potential and Stimulation Currents (2 plots), and the Calcium-Dependent Potassium Current, Conductance, and Gate (3 plots).***

Insert a screenshot of the simulation and label the figure.

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How does the time constant of calcium buffering affect the duration of the calcium-dependent potassium current? Please press the Ca and Ca-dependent K currents again. Change the Total duration to 300 ms. Run the simulation and *measure the duration of the afterhyperpolarization in the top voltage trace.* ***Take a screenshot of the Membrane Potential (1 plot).***

Insert a screenshot of the simulation and label the figure.

Now change the value of the Calcium buffering time constant from 25 ms to 100 ms. Run the simulation. *What do you observe about the duration of the afterhyperpolarization? Explain* in terms of the calcium buffering and the calcium-dependent potassium current. ***Take a screenshot of the Membrane Potential (1 plot).***

Insert a screenshot of the simulation and label the figure.

Now change the value of the Calcium buffering time constant to 6 ms. Run the simulation. *What do you observe about the duration of the afterhyperpolarization? Explain* in terms of the calcium buffering and the calcium-dependent potassium current. ***Take a screenshot of the Membrane Potential (1 plot).***

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Persistent Sodium Current

Question 8: Run the persistent sodium simulation and explain your observations.

There are multiple kinds of sodium channels. In this simulation, you can study one of them, the persistent sodium current, which (unlike the more usual sodium current that contributes to the action potential) takes a very long time to inactivate.

Please press the button Persistent sodium current. Note that no current is now injected into the model neuron. *What do you observe? Explain* what is happening in terms of the graphs of the Persistent Sodium Current, Conductances, and Gates. ***Take a screenshot of the Membrane Potential and Stimulation Currents (2 plots), and the Persistent Sodium Currents, Conductances, and Gates (3 plots).***

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Now, under Current Clamp, please set the Stimulus delay to 1 ms, the Pulse duration to 30 ms, and the Number of pulses to 1, and please set the Persistent sodium conductance to 0 μS. Run the simulation. *How does the neuron respond?* ***Take a screenshot of the Membrane Potential and Stimulation Currents (2 plots), and the Hodgkin-Huxely Currents, Conductances, and Gates (3 plots).***

Insert a screenshot of the simulation and label the figure.

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Now restore the Persistent sodium conductance to 0.05 μS. *How does the neuron respond? Please explain* both in terms of the persistent sodium conductance and in terms of the Hodgkin Huxley gates. *Which of these gates is most likely to account for the observations?* ***Take a screenshot of the Membrane Potential and Stimulation Currents (2 plots), the Hodgkin-Huxely Currents, Conductances, and Gates (3 plots), and the Persistent Sodium Currents, Conductances, and Gates (3 plots).***

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Fast Potassium Current

Question 9: How does the cell in the fast potassium simulation respond to a short and longer stimulus?

There are a wide variety of other potassium conductances, which play a variety of important roles in shaping the behavior of nerve cells. We will explore one of them, known as the fast potassium or IA conductance.

Please press the Fast potassium current button. *What happens during the current pulse?* Under the Current Clamp menu, please increase the Pulse duration to 3 ms. *What happens now during the current pulse? Please explain*, using the graphs of the Fast Potassium Current, Conductance, and gates. ***Take a screenshot of the Membrane Potential and Stimulation Currents (2 plots), and the Fast Potassium Currents, Conductances, and Gates (3 plots).***

Insert a screenshot of the simulation and label the figure.

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The IA conductance, unlike the delayed rectifier potassium current of the Hodgkin Huxley model, inactivates, and this inactivation can be removed through hyperpolarization. Under Current Clamp, please set the Stimulus current first pulse to 0, the Pulse duration to 100 ms, Inter-stimulus interval to 0, and the Number of pulses to 2. Change the Total duration to 250 ms. Run the simulation. *How many spikes do you observe during the depolarizing current pulse?* ***Take a screenshot of the Membrane Potential and Stimulation Currents (2 plots), and the Fast Potassium Currents, Conductances, and Gates (3 plots).***

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Insert a screenshot of the simulation and label the figure.

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Now, change the Stimulus current first pulse to -0.5 nA, and again run the simulation. *How many spikes do you observe during the depolarizing current pulse? Please explain* what has happened, again making reference to the graphs of the Fast Potassium Current, Conductance, and gates. ***Take a screenshot of the Membrane Potential and Stimulation Currents (2 plots), and the Fast Potassium Currents, Conductances, and Gates (3 plots).***

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All Together

Question 10: What is the functional significance of having so many different conductances?

One important feature of nerve cells is their ability to respond with different firing frequencies to a given sensory input. We can explore this using the simulation.

Please press the Simple simulation button. Set the Stimulus current first pulse to 0.5 nA, the Pulse duration to 180 ms, and the Total duration to 200 ms. Run the simulation. *How many action potentials do you observe? What is the period between the first and the second, and between the last two?* ***Take a screenshot of the Membrane Potential and Stimulation Currents (2 plots)*** **You can measure the period from peak to peak, trough to trough, or at the half-width, but you must explain how you did it.**

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Now add in all the currents that we have examined separately by pressing the Full simulation button. Again, set the Stimulus current first pulse to 0.5 nA, the Pulse duration to 180 ms, and the Total duration to 200 ms. Run the simulation. *How many action potentials do you observe? What is the period between the first and the second, and between the last two?* ***Take a screenshot of the Membrane Potential and Stimulation Currents (2 plots)***

Insert a screenshot of the simulation and label the figure.

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Please explain how the different conductances in the full simulation contribute to this result. Which current or currents are most important? Please test your hypotheses by reducing the conductance of a single current, and examining the effects. Then restore the conductance to its original value, before altering a second conductance. **Show whichever pictures are necessary to prove your hypothesis.** You will know that you understood the unit if you can answer this question.

(format this however you would like, please remember to show pictures)

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Discussion: