Simulation Exercises and Homework Questions – Ion gradients and Ion channels

The simulations you will perform in class use the Electrophysiology of the Neuron simulation software. This software simulates whole-cell patch-clamp experiments and it provides very realistic simulations of what the outcomes of actual experiments would look like.

Begin by running the SimCC program. This simulates patch-clamp experiments in the "current-clamp mode", which is to say that the experimental configuration allows you to deliver current to the cell while recording the voltage of the membrane.

1. Simulations: Resting membrane conductances and resting potential. Download and open the file REST.CC5 simulation file from the class website.

Now, select Parameters | Ions and note the extracellular and intracellular concentrations of K^+ and Na^+. Use these values to calculate E_K and $E_{NA.}$ NOTE: The program assumes a temperature of 35 °C so you will need to use a value of 26.55 for RT/F

Select Run | Begin. This will produce one "sweep" of data that shows the resting potential of your neuron.

Go to Parameters | Conductances and note the numbers for pKleak and PNaleak. These are the resting membrane conductances for K^+ and Na^+ ions (representing the relative number of K^+ and Na^+ ion channels open at rest).

Set pKleak to 0 (making the membrane permeable only to Na^+) and select Run | Overlay.

Now, set pKleak back to 1 and set pNa leak to 0 to make the membrane permeable only to K^+ and select Run | Overlay.

Question 1a: What were your calculated values for EK and ENa?
Question 1b: At what voltage does the membrane potential stabilize when you set pKleak to 0? Why does the membrane potential change to this value?
Question 1c: At what voltage does the membrane potential stabilize when you set pNaleak to 0? Why does the membrane potential change to this value?

2. Simulations: Ion concentrations and resting potential.

Set the simulation back to its starting point by setting pKleak to 1 and pNaleak to 0.06. Now, answer the following questions by changing the intracellular and extracellular concentrations of K^+ and Na^+. To do this select Parameters | Ions and change the appropriate values before running each sweep of data.

Question 2a: What happens when you increase extracellular K^+ concentration? Why does this happen?
Question 2b: What happens when you eliminate the K^+ gradient by setting the intra- and extracellular K^+ concentrations to the same value? Why does this happen?
Question 2c: What happens when you double the original extracellular K^+ concentration from 3.1 to 6.2 mM? What happens when you go back to the original K^+ concentration then double the intracellular Na^+ concentration. What explains the small effect of changing Na^+ concentration compared to changing K^+ concentration?

3. Simulations: Voltage clamp of isolated Na^+ and K^+ currents

Close the SimCC program and run the program SimVC. This simulates patch-clamp experiments in the "voltage-clamp mode", which is to say that the experimental configuration allows you to control the membrane voltage while recording the current that moves across the membrane.

Download and open the file Na_K.VC5 this file simulates a cell with voltage-gated K^+ channels and voltage-gated Na^+ channels and the protocol runs a single sweep where the membrane voltage steps from -100 mV to 0 mV, thereby activating both Na^+ and K^+ channels. Run a single sweep of data to see the membrane current that results from the voltage step to 0 mV.

Now, knowing what you know about equilibrium potentials – find a way to manipulate those equilibrium potentials to isolate just the Na^+ current and then to isolate just the K^+ current. (Another way to think about this is how would you eliminate the Na^+ current or eliminate the K^+ current).

Overlay a sweep of isolated Na^+ current (I_{Na}) and a sweep of K^+ current (I_K). Show this to your instructor to prove your group's awesomeness.

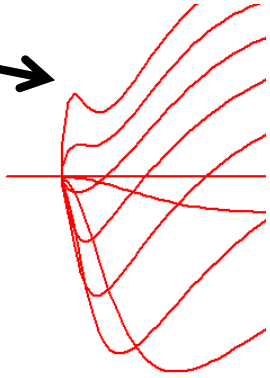
Question 3a: How did you eliminate the Na^+ current and then the K^+ current. Why does this work?
Question 3b: Describe as many differences as you can between the Na^+ current and the K^+ current.

4. Simulations: Advanced voltage clamp

Open the file NA_K_IV.VC5 - this file simulates a cell with voltage-gated K^+ channels and voltage-gated Na^+ channels. Begin by running the full protocol – selecting Run | Begin. Note that the simulation runs ten sweeps of data in quick succession.

You can mimic the effects of blocking sodium channels with the neurotoxin Tetrodotoxin (TTX) by setting gNa to 0 under Parameters | Conductances. You can also mimic the effects of blocking potassium channels with α-Dendtrotoxin (a snake venom toxin) by setting gK to 0.

Now, using your newfound skills and knowledge, figure out what causes the weird hump at the beginning of some of the current traces?



Question 4a: What causes the weird hump at the beginning of some of the current traces?
Question 4b: Why does this happen?

Final step: Isolate only INa and produce an IV plot for this current. Do this by running the simulation, then selecting Epoch measurement in the upper left window. Set "polarity" to 0 so the IV graph plots the absolute peak of the currents. Set epoch measurement to 1, and check the "visible" box to display the IV plot at the bottom of the graph window. Estimate E_{Na} from your plot. What is the activation voltage for INa? At what voltage does INa reach its maximum? Show your results to your instructor to verify that your results are accurate.

HOMEWORK ASSIGNMENT:

Submit your answers to questions 1-4 via Canvas. Be sure to use figures from your simulations to support your answers where appropriate.