

Part I: Maintaining the Resting Potential

Introduction:

Ions Play an Essential Role in Neuron Signaling

All living cells—including nerve cells—maintain a difference in the concentration of ions across their membranes. These ions play an essential role in neuronal signaling. A small excess of positively charged ions on the outside of the neuronal membrane results in a negatively charged cell interior relative to the outside environment. This charge difference—or voltage—is called the **membrane potential** and is measured with a voltmeter. A membrane exhibiting membrane potential is said to be **polarized**. When the neuron is not sending a signal, the (resting) voltage ranges from -60 to -80 mV (millivolts). The membrane potential at rest is called the neuron's **resting potential**. For this lab activity, we will consider a neuron with a resting potential of -70 mV.

Ion transport mechanisms in the neuron's cell membrane produce and maintain the slight ionic imbalance across the neuron's cell membrane. The permeability characteristics of a neuron's cell membrane are, in part, determined by specific membrane transport channels. Channels in the cell membrane that transport negatively charged ions are relatively few in number or are closed and not displayed in this model.

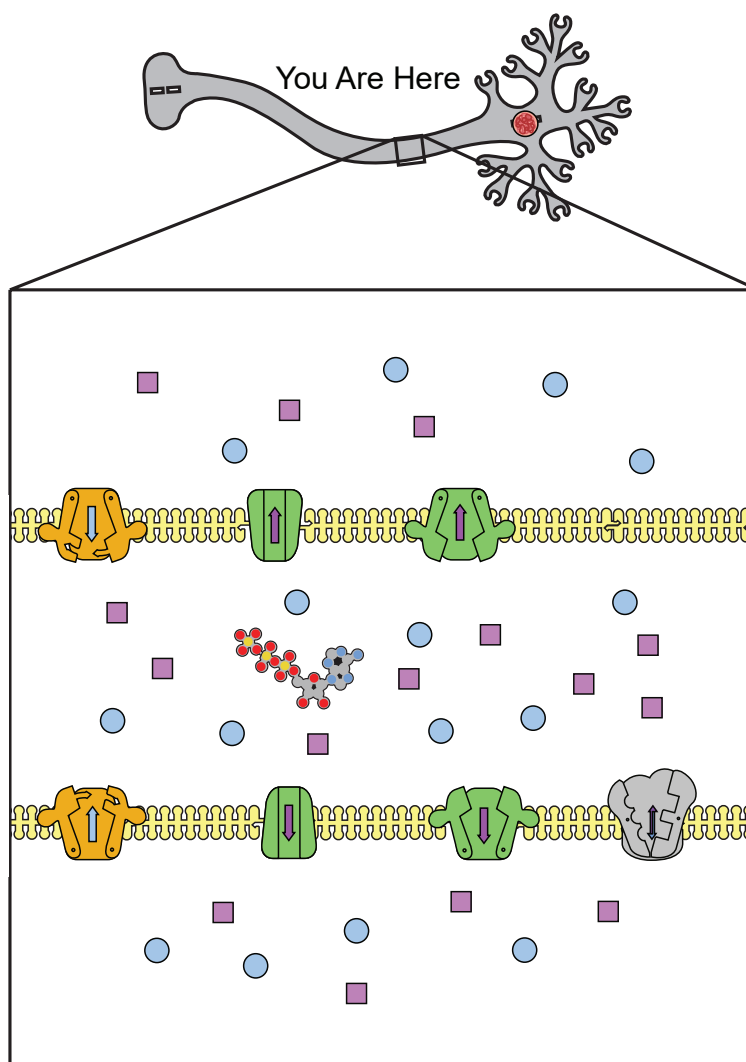
Table 1: Key Ion Concentrations of Mammalian Neurons

Ion	Extracellular Concentration (mM)	Intracellular Concentration (mM)
Potassium (K^+)	5	140
Sodium (Na^+)	150	15
Chloride (Cl^-)	120	10
Large anions (A^-), such as some proteins, inside the cell	Not applicable	100

Note that in most neurons, the concentration of potassium ions (K^+) is higher inside the cell, while the concentration of sodium ions (Na^+) is higher outside.

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Set up the membrane of the axon of a neuron according to the diagram below:



1a. Label the neuron membrane, the voltage-gated sodium channel, the potassium leak channel, the voltage-gated potassium channel and the sodium-potassium pump in the diagram above.

To model this scenario, place 7 Na^+ (round blue pieces) and 8 K^+ (square purple pieces) inside of the cell to simulate the intracellular environment ion concentrations. Place 8 Na^+ and 7 K^+ outside of the cell to simulate the extracellular ion concentrations. Place the ATP molecule on the inside of the model cell. (Keep in mind that you are constructing a **model** and that in reality there are more than 7 sodium ions and 8 potassium ions inside the cell!)

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1b. In a resting neuron, what positive ion is most abundant outside of the plasma membrane

1c. The K^+ gradient dictates that potassium ions will flow in what direction?

1d. What happens to the resting potential of the cell when K^+ ions move?

1e. What direction does the concentration gradient dictate the Na^+ ions flow?

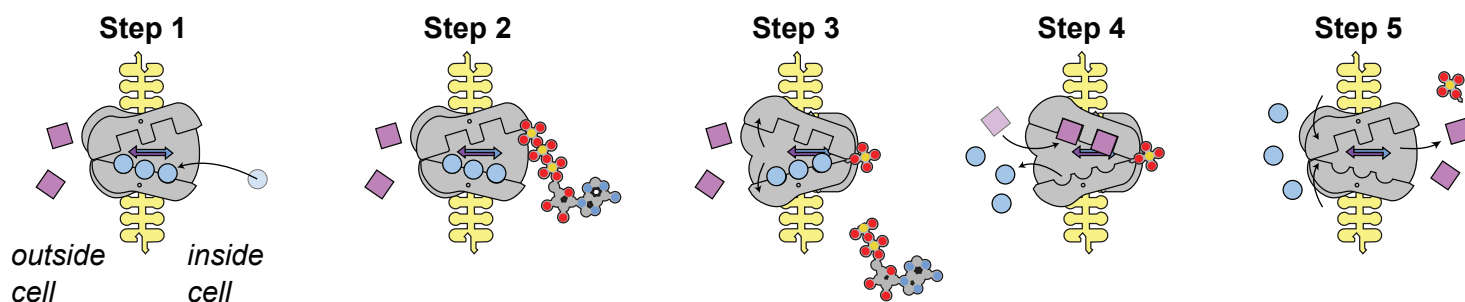
The sodium-potassium pump uses energy in the form of ATP to move these ions against their concentration gradients and establish and maintain the *normal* intracellular ion concentrations.

Set the sodium-potassium pump so that it is open to the inside of the neuron.

2a. Record the initial ion concentrations in your model in the table provided below:

Ion Types	Initial Amount	Amount After First Cycle	Amount After Second Cycle
Intracellular (Na^+)			
Intracellular (K^+)			
Extracellular (Na^+)			
Extracellular (K^+)			

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Step 1: Bind three intracellular sodium ions to the appropriate spots in the protein.

Step 2: Bring the ATP in close proximity to the pump. Sodium ion binding stimulates **phosphorylation** of the pump protein by ATP. In other words, a phosphate group is added to the sodium-potassium pump from the ATP molecule. Bind the phosphate group of the ATP to the sodium-potassium pump.

Step 3: Phosphorylation causes a change in the shape of the protein. You can demonstrate this by “swinging” the sides of the protein so that it opens to the outside of the cell.

Step 4: The shape change reduces the protein’s binding affinity for sodium ions and increases the binding affinity for potassium ions. Remove the sodium ions from the protein and deposit them outside the cell and bind two potassium ions to the appropriate spots in the protein.

Step 5: Potassium ion binding triggers the release of the phosphate group from the protein. Loss of the phosphate group results in the restoration of the protein’s original shape which then releases the potassium ions. Swing the sides of the protein back so that they open to the inside of the cell and deposit the potassium ions.

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2b. Record the ion concentrations in the table on page 3 after completing the first cycle of the action of the sodium-potassium pump.

2c. What is the initial overall positive charge inside the cell compared to the outside the cell?

2d. Compare the total intracellular positive charge to the total extracellular positive charge after the first cycle of the sodium-potassium pump.

2e. Repeat a second cycle of the sodium-potassium pump. Record the ion concentrations after completing the second cycle in the table on page 3.

2f. What is the difference in charge between the inside and outside of the neuron membrane after the second sodium-potassium pump cycle?

2g. What can you conclude about the difference in charge across the membrane after two cycles of the sodium-potassium pump?

2h. Compare the initial number of Na^+ on the outside of the cell to the final number of Na^+ on the outside of the cell after the second cycle of the sodium-potassium pump.

2i. Compare the initial number of K^+ on the inside of the cell to the final number of K^+ on the inside of the cell after the second cycle of the sodium-potassium pump.

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2j. Why is ATP required in this process?

A resting neuron has many open potassium channels (potassium leak channels) but very few open sodium channels. As a result, Na^+ and other ions can't readily cross the membrane. As you can see from Table 1, the concentration of K^+ is 140 mM inside the cell but only 5 mM outside. This chemical concentration gradient drives a net outflow of K^+ through the potassium leak channels. The movement of positive charges from the inside of the neuron to the outside of the neuron contributes to the difference in electrical charge across the membrane. Move a few more of the potassium ions through the potassium leak channels from the inside of the neuron to the outside.

Eventually, the extracellular buildup of positive charge will oppose the flow of additional positively charged potassium ions out of the cell. In other words, the electrical gradient counterbalances the chemical concentration gradient for potassium ions. The net flow of K^+ proceeds until the chemical and electrical forces are in balance. When this balance has been achieved, the neuron has reached its **resting potential**.

3a. Why do you think the sodium leak channels are not shown in this model?

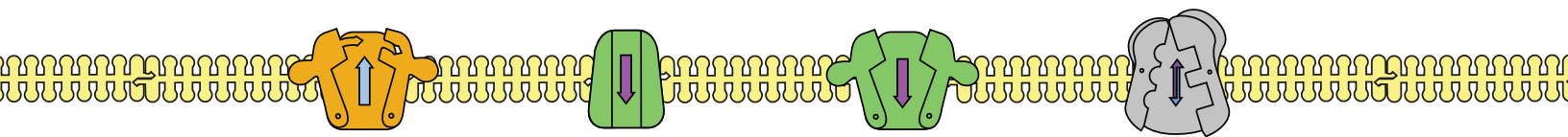
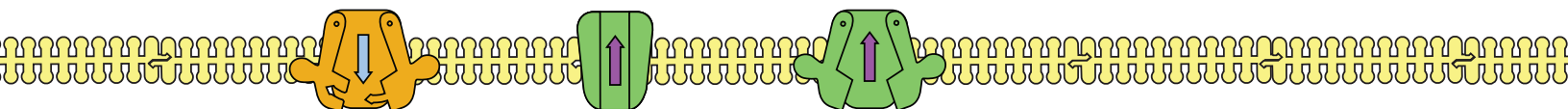
3b. Identify and describe the forces that contribute to the extracellular concentrations of Na^+ and K^+ compared to intracellular concentrations of Na^+ and K^+ .

3c. When the neuron is at resting potential, where is the higher concentration of positive charges located?

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A membrane that exhibits a resting membrane potential is said to be **polarized**. The sign of a membrane's voltage indicates the charge on the inside surface of the polarized membrane. For example, -70mV indicates the potential difference has a magnitude of 70 mV and that the inside of the membrane is negative with respect to the outside surface. The relative difference in charge is noted by using a negative for the inside of the neuron and a positive charge for the outside of the neuron.

4. Label the positive and negative differential in the neuron membrane below if the voltage reads +30 mV. Explain your answer. _____



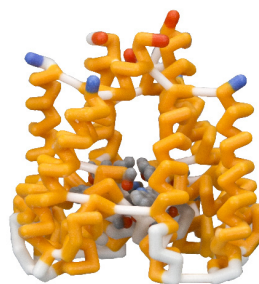
Discuss: How is a polarized *molecule*—like water—different than a polarized *membrane*?

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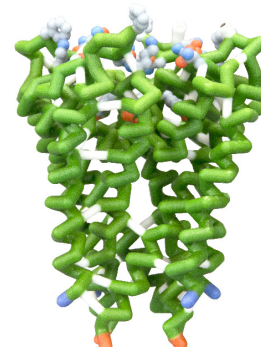
Optional Activity: Comparing the Voltage-Gated Sodium Channel to the Voltage-Gated Potassium Channel

1i. Observe differences in the channels.

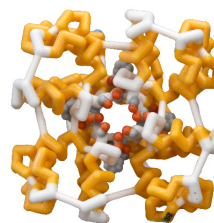
1j. Propose a mechanism for how the voltage-gated potassium channels distinguish sodium ions from potassium ions.



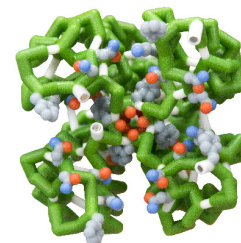
*Sodium Channel
Mini Model*



*Potassium Channel
Mini Model*



*View of the Na⁺
Channel Pore*



*View of the K⁺
Channel Pore*

1k. What structural differences can you observe in the channel pore of these voltage-gated channels?
