Biology 12

ACTION POTENTIAL SIMULATION

BACKGROUND:

The plasma membrane of cells is a selectively permeable barrier, which separates the internal contents of the cell from the surrounding extracellular fluid. While hydrophobic molecules and small, polar uncharged molecules such as water and carbon dioxide can pass freely across the membrane, the plasma membrane is largely impermeable to ions and large polar molecules. Special mechanisms are required to move these latter types of molecules across the plasma membrane.

Many types of large negatively charged molecules such as proteins and DNA reside within the cell. The interior of a cell also contains a much higher concentration of potassium ions (K^+) than the extracellular fluid, and a much lower concentration of sodium ions (Na^+) as compared to the exterior concentration. As a result of these differences, the interior of the cell is more negatively charged than the exterior.

As an analogy, think of the plasma membrane as a barrier separating two solutions; the cytoplasm on one side of the barrier and the extracellular fluid on the other side. As described above, these two fluids have different concentrations of charges. The difference in the charges between the two solutions is called a **POTENTIAL DIFFERENCE**. Since the charge difference occurs across a membrane, it is referred to as a **MEMBRANE POTENTIAL**. Each cell in an organism has a membrane potential, but only nerve cells and muscle cells use this potential to generate an electrical impulse.

When the nerve or muscle cell is not generating electrical impulses, it is at rest and the difference in electrical charge across the membrane is called the **RESTING MEMBRANE POTENTIAL**. The magnitude of the resting membrane potential can be measured with two electrodes and an oscilloscope, which essentially functions as a quickly responding voltmeter. One electrode is inserted into the cell, the other into the extracellular fluid. A typical nerve cell has a resting potential of about –70 millivolts (mV). The negative sign indicates that the interior of the cell is negative with respect to the exterior.

To initiate and propagate a nerve impulse, there must be a change in the resting membrane potential. To accomplish this, there must be a change in the electrical makeup of the interior of the cell as compared to the exterior. Sodium and potassium ions are relatively impermeable to the cell membrane in its existing state. However, upon receipt of a stimulus (touch, heat, light, etc.) specialized channels for sodium or potassium embedded within the plasma membrane will open, and these ions will flow down their electrochemical gradients (a variation on diffusion, which includes a consideration of charge separation as well as molecules flowing from an area of high to low concentration). The change in the membrane potential triggers an **ACTION POTENTIAL**, which propagates the nerve impulse along the length of the axon of the nerve cell.

SUMMARY OF THE EVENTS WHICH TRIGGER AN ELECTRICAL IMPUSLE IN NERVE CELLS

(see figures on the following page)

STAGE A – Cell is in resting state. Sodium and potassium gates are closed. Resting membrane potential is around –70 mV.

STAGE B – Stimulus triggers changes in nerve cell membrane = **DEPOLARIZING PHASE**

1) Voltage-gated sodium channels open and sodium ions begin to flow in, down their concentration gradient. Potassium channels remain closed.
2) The influx of sodium ions changes the membrane potential to about –55 mV. At this point, an action potential is triggered (the process goes to completion – **ALL-OR-NONE RESPONSE** – if –55 mV is not reached, no action potential occurs)
3) Many more voltage-gated sodium channels open. The inward rush of positively charged sodium ions causes a depolarization of the membrane, to around +40 mV.

STAGE C – REPOLARIZING PHASE

1) Sodium inactivation gates close the sodium channels and the voltage-gated potassium channels open.
2) Potassium ions flow out of the cell through the open gates. This efflux of positive charges begins to restore the membrane potential to resting state.

STAGE D – REFACTORY/RECOVERY PERIOD (UNDERSHOOT)

1) Sodium channels are closed and are maintained closed due to the repolarization. However, potassium channels are slow to respond to repolarization, so "too many" potassium ions flow out and the membrane potential briefly is more negative than the resting potential of –70 mV.
2) The distribution of sodium and potassium is reversed and is restored by the simultaneous decline in sodium permeability to it s resting level and increase in potassium permeability to a higher than normal level. (Thus, these permeability changes result from actions of membrane channel proteins, not pump proteins.)

STAGE E – (return to "A") **Resting state**. With resting membrane potential and ionic distribution restored, the cell is ready to fire another impulse. The sodium-potassium pump works in the resting state to help maintain the distribution of ions (sodium on the outside and potassium on the inside of the membrane).

THIS ENTIRE PROCESS TAKES, ON AVERAGE, ABOUT 3 MILLISECONDS.

Features of Na/K Pump Proteins

- Pump proteins are found in virtually all cell types of all species. The complete amino acid sequence of pump proteins has been determined. Strong homologies exist in the amino acid sequences of very diverse species, such as bacteria, fish, sheep, etc.

- It is estimated that a single neuron may have as many as one million pump molecules.

- Outward pumping of sodium is coupled, as an exchange, with inward pumping of potassium.

- The rate of ion transport (pumping) across a membrane is very, very slow compared to the rate the ions diffuse through membrane channel proteins.

  [**NOTE**: In one second, one pump protein pumps about 300 Na^+ out of the cell, and 200 K^+ into the cell while hydrolyzing 100 ATP molecules]

- Under resting conditions, pumping activity may consume up to 30% of a nerve cell's ATP supply. When nerve cells are very active, its percentage my be much higher.

- Pumping plays an important role in controlling cell volume and in maintaining the observed Na^+ and K^+ concentrations across membranes.

- Pumping is NOT the basis for rapid electrical signaling in nerve cells. Pumps act much too slowly to contribute to the membrane potential changes seen during an action potential.

Features of Voltage-Gated Sodium Channel Proteins

- Sodium channels have moveable gates that respond to depolarization (increased positivity inside cell). All gates in a channel must open for the channel to be open and for Na^+ to diffuse through it.

- Sodium channels have activation gates (*"m" gates*) and inactivation gates (*"h" gates*). Under resting conditions, *"m"* gates are normally closed and *"h"* gates are normally open.

- Depolarization tends to make each "m" gate open, and this happens rapidly. Opening of *"m"* gates leads to depolarization.

- Depolarization tends to make each *"h"* gate close, and this happens more slowly. Closing of "h" gates contributes to repolarization.

- Typically, a Na+ channel opens up and then closes again, all in a millisecond, or less.

- The open channel can be though of as a selective pore through which Na^+ ions rapidly diffuse.

  [**NOTE**: The water molecules that are attached to each Na^+ ion are probably stripped off as the ion passes through the open channel.]

- When open for one millisecond ($1/1000^{th}$ of a second), it is estimated that a single channel allows passage of about 10 000 Na^+ ions. This is more that 30 000 times greater than the rate that Na^+ is moved across the membrane by pumping!!

SETTING UP THE SIMULATION

MATERIALS:

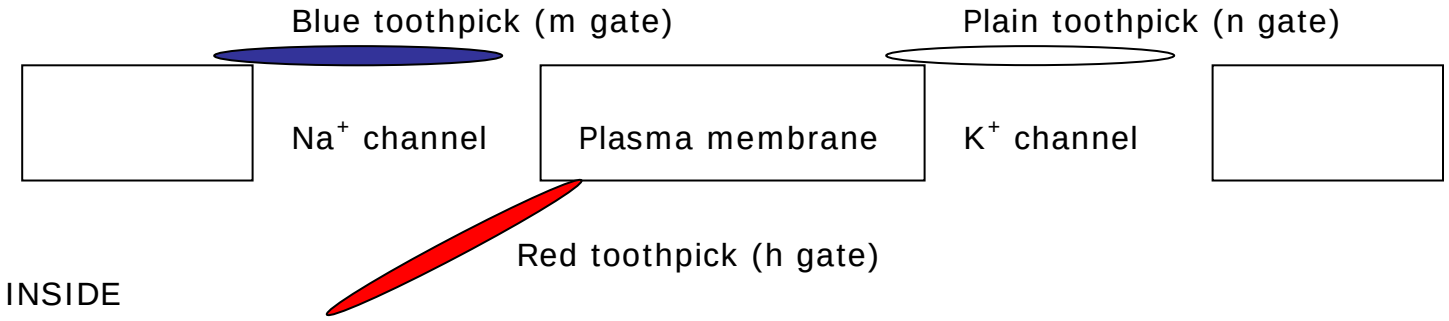
- 1 sheet of poster board
- 1 zip-lock storage box containing the following:
- 3 toothpicks (1 red, 1 blue, 1 plain)
- 20 spiral pastas (represent negatively charged large proteins and DNA)
- 35 black beans (represent sodium ions, Na^+)
- 35 red beans (represent potassium ions, K^+)
- 35 white beans (represent chloride ions, Cl^-)
- marker
- chart for tallying charges

For steps 1 – 3, refer to the drawing below:

1. Across the center of the poster board, draw a plasma membrane with two gaps in it. Make the membrane about 3 cm thick; make each gap about 7 cm wide. Above the membrane, along one side, write "OUTSIDE" (representing the extracellular fluid); below the membrane write "INSIDE" (representing the axoplasm).

2. Place the blue toothpick horizontally above the left gap, overlapping slightly with the membrane to the left of the gap. Place the red toothpick at a 45° angle below the left gap, with the right end of the toothpick overlapping slightly with the membrane to the right of the gap. These toothpicks simulate a voltage-gated sodium channel, with the blue toothpick serving as the activation (or *m*) gate and the red toothpick the inactivation (or *h*) gate.

3. Place the plain toothpick horizontally above the right gap, overlapping slightly with the membrane to the left of the gap. This toothpick and gap simulate a voltage-gated potassium channel.



4. Below the membrane, in the area representing the cytoplasm INSIDE of the cell (axoplasm), place the following:
   - 3 black beans = 3 sodium ions (Na^+)
   - 30 red beans = 30 potassium ions (K^+)
   - 2 white beans = 2 chloride ions (Cl^-)
   - 20 pasta spirals = large anions (A^-) – proteins and DNA

5. Above the membrane, in the area representing the extracellular fluid OUTSIDE the cell, place the following:
   - 30 black beans = 30 sodium ions (Na^+)
   - 1 red bean = 1 potassium ion (K^+)
   - 24 white beans = 24 chloride ions (Cl^-)

CARRYING OUT THE SIMULATION

6. First, observe the difference in the distribution of beans on the inside and outside of the membrane.

7. Count up the charges inside and outside the "cell". Each bean represents one charge and each pasta spiral represents 2 charges. Sodium and potassium are positive charges; chloride and the large anions are negative charges.
   NOTE: the anions (pasta) each receives two charges even though they represent large molecules with many negative charges. Two is easier to work with and still shows the correct overall pattern.

8. Determine the absolute value in differences of charge between the inside and the outside – the "charge separation" of the two sides of the plasma membrane. Record your count and calculations in the first column of the chart (Resting state or Resting Membrane Potential). Think of the numbers on a number line to help visualize this concept.

9. Now initiate a STIMULUS!
   A) Voltage-gated sodium channels in the plasma membrane open. To simulate this, raise the right end of the blue toothpick (activation gate) until it is about a 45° angle from the membrane, thus opening the channel.
   B) Sodium ions will now diffuse across the membrane. (Think: Which way will they go? Move 15 sodium ions in the proper direction across the membrane. You have just depolarized the cell)

10. This change in charge is the DEPOLARIZATION PROCESS. Calculate the change in membrane potential caused by this shift by filling in column 2 on the chart.

11. The depolarization process then causes the voltage-gated potassium channels in the plasma membrane to open and the sodium channels to close. To simulate this, raise the right end of the plain toothpick until it is about at a 45° angle from the membrane, thus opening the channel. Close the sodium channel by moving the red toothpick (inactivation gate) up into a horizontal position below the left-hand gap. Which way will potassium diffuse? Move 15 potassium ions (red beans) in the proper direction across the membrane. Then close the potassium channel by lowering the plain toothpick again.

12. Calculate the change in membrane potential caused by this shift. Explain what the repolarization process has done to your set up. Be specific!

13. Is the distribution of ions the same as in the initial resting state? If not, how is it different? Now use ACTIVE TRANSPORT to move the sodiums and the postassiums back to their initial resting state. The time it takes to do this simulates the refractory period.

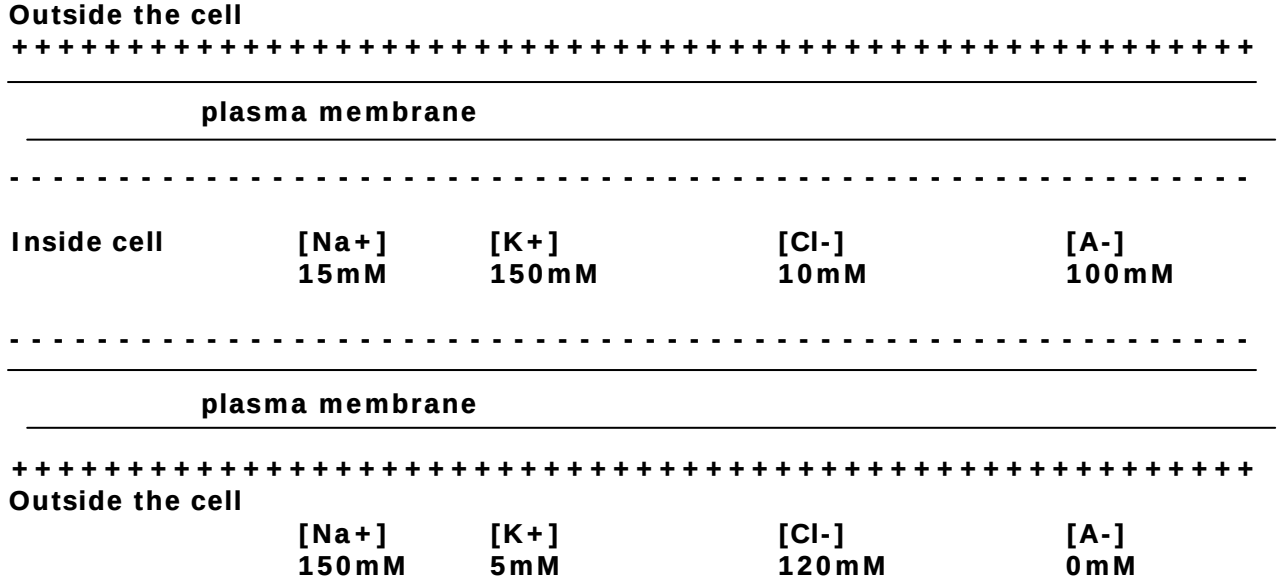
14. Add up the charges and concentrations to be sure that all is well in the resting state.

WHAT IS GONG ON? Action potential simulation

The ratios of the beans used in this exercise are accurate ratios of the ions in the resting state of a typical nerve cell and its extracellular fluid with one bean is equal to 5mM (millimolar) concentration. (See figure below):

Ion Distribution in Resting Membrane Potential

Millimolar concentrations [mM] of the major ionic components differ within and outside the cell and are the basis of the cell's membrane potential.



The ions described here all have a charge of one, except for the anions. DNA has a negative charge on each phosphate group, so a single molecule would have billions of negative charges. Proteins and other large molecules, which compose the "anion" category, also may contain multiple negative charges. We represent (not to scale) these large molecules by using the spiral pasta, larger than the beans, and give it a –2 charge.

When the resting potential is calculated, you will see that the interior is –9, the exterior is +7. The absolute difference between these numbers is 16, with the interior being more negative; modeling the membrane potential, we can call it –16 as the membrane potential.

As the voltage-gated sodium channels open due to a stimulus, sodium ions diffuse into the cell. We let half go in (15) which leads to equilibrium in concentrations. When the charges are again added p, we see that removing 15 positive charges from the outside and adding them to the interior means that the external charge is now –8, and the interior charge is +6. The absolute value of the difference is 14. To relate this to the membrane potential, we say it is +14. Just as in the real nerve cell, the depolarization results in a reversal of the charge of the membrane potential.

When the depolarization causes the voltage-gated sodium channels to close and the voltage-gated potassium channels to open, potassium diffuses out of the cell. We move 15, and find that this redistribution results in repolarization, as well as the original membrane potential of –16.

Even though the resting membrane potential had been restored, there has been a reversal in the concentration of sodium and potassium ions. Before another action potential could occur, the proper balance must be restored. 15 sodiums are moved from the interior of the cell to the

exterior, and 15 potassiums are moved from the exterior to the interior. In nature, this process is accomplished by changes in permeability to sodium and potassium ions, with a decline in Na^+ permeability to its resting level and an increase in K^+ permeability to a higher than normal level. The length of time it takes to restore this balance is the refractory period.
With the ionic and charge balances restored, the cell is ready to accept another stimulus and trigger and action potential.

It is important to note that only one small area of a nerve cell depolarizes at on time. To propagate the nerve impulse the length of the cell, from the dendrites to the axons and across the synapse, each successive region must be depolarized, not unlike dominoes falling in a cascade. The depolarization of one region triggers the depolarizing of the adjacent region, and so on. The ionic exchange occurs in successive regions of dense concentrations of voltage-gated sodium and potassium channels, called NODES OF RANVIER.

This simulation is accurate through the repolarization phase of action potentials. It breaks down at the active transport phase needed to restore the correct distribution of sodium and potassium ions, and does not account for the differential diffusion of sodium and potassium ions due to some “leakiness’ of the membrane.

Credits:
This activity is a modification of one presented at the 1997 NABT Convention in Minneapolis, MN by Karen E. Kalumuck, of the San Francisco Exploratorium. The “Background” and “What’s Going On” sections are still largely her information, edited and modified slightly by Martha Friedlander, Woodrow Wilson Neurobiology TORCH Team Leader. The actual simulation is modeled after one presented by Ms. Kalumuck, but with entirely new materials and several additions, most notably the voltage-gated channels in the membrane; it was redesigned by Martha Friedlander. The diagram illustrating the “Opening of Voltage-gated Channels during an Action Potential" and the page listing features of voltage-gated channels and pump proteins are taken from material presented by Dr. Charles Drewes of Iowa State University at the 1996 Woodrow Wilson Biology Institute at Princeton University and a the 1997 convention in Minneapolis, MN.

SUMMARY: ACTION POTENTIAL MODEL WITH BEANS

TYPE OF ION	BEAN / PASTA MODEL	CHARGE
Large anions (A^-)	Spiral pasta	-2
Sodium (Na^+)	Black beans	+1
Potassium (K^+)	Red beans	+1
Chloride (Cl^-)	White beans	-1

		RESTING STATE		DEPOLARIZATION (Na^+ gates open)		REPOLARIZATION (Na+ gates close / K+ gates open)	
	Ion	#	Total charge	#	Total charge	#	Total charge
INSIDE	A^-						
	Na^+						
	K^+						
	Cl^-						
Sum of all charges inside cell:							
OUTSIDE	A^-						
	Na^+						
	K^+						
	Cl^-						
Sum of all charges outside of cell:							
Charge separation WRT cell interior							

***NOTE**: Don't forget that to reach correct ionic concentrations for the resting membrane potential the positions of the sodium and potassium ions must be reversed. In nature, this process is accomplished by changes in permeability to sodium and potassium ions, with a decline in Na^+ permeability to its resting level and increase in K^+ permeability to a higher than normal level. These changes are accomplished by the action of membrane channel proteins.*

Chart designed by Karen E. Kalumuck, San Francisco Exploratorium and edited by Martha Friedlander