SynapseLAB exercises

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Using the application

This version of SynapseLAB is a standalone HTML file. It does require JavaScript but does not require an Internet connection.

To use the application:

1. Download synapseLab.html from the GitHub page where you got this manual
2. Open it using a web browser – Firefox, Chrome, Edge/Explorer
3. Follow the prompts on the screen and use this guide for ideas.

Nernst and Resting Membrane Potentials

Theory

1. Take a look at the table of values in the upper left corner. Concentrations of four different ions are given for both intracellular and extracellular regions. Additionally, each ion has a value called *g*, or permeability. This value reflects the ability of the ion to cross the cell membrane, or the number of **leak channels**. Note that K has the vast majority of open leak channels at rest, and so it has the highest permeability.
2. The Nernst equation gives the **reversal potential** for a given ion. This is the voltage at which the ion would be at equilibrium, i.e., with no net movement across the membrane. It is derived from setting the diffusion and electrical potential equations equal to each other, so that the electrical and chemical gradients balance. At human body temperature for a cation,

$$V_{ion} = -61.5\ mV \times \log\frac{[\text{ion}]_{outside}}{[\text{ion}]_{inside}}$$

   The -61.5 mV comes from a term in the original Nernst equation, -RT/zF, which also includes temperature (T) and ionic charge (z) and the conversion factor from natural to base 10 log. Note that for an anion, you would need to switch the concentrations of the ion inside and outside to make the equation accurate.

3. The Goldman equation gives the **resting membrane potential (RMP)** for the **entire cell**. It combines the Nernst equation for each ion, but weighted by the permeability. This is the actual voltage measured across the cell membrane at rest.

$$E_{cell} = -61.5\ mV \times \log\frac{g_{Na}[\text{Na}]_{out} + g_{K}[\text{K}]_{out} + g_{Cl}[\text{Cl}]_{in}}{g_{Na}[\text{Na}]_{in} + g_{K}[\text{K}]_{in} + g_{Cl}[\text{Cl}]_{out}}$$

4. Note that Cl is “reversed” relative to K and Na in the Nernst and Goldman equations with respect to intracellular/extracellular concentrations. This is because it has a negative charge while K and Na are both single-positive charges.

Questions for the RMP and Nernst equation module

Some conceptual questions before touching the buttons

1. Note the permeability values for K, Na, Cl, Ca. What channels or pumps probably contribute to each value? Why would the cell keep Ca permeability very low?
2. What protein creates and maintains the Na/K gradients?
3. Are each of the following more concentrated on the inside or the outside of the cell?
   - Na
   - K
   - Cl
   - Ca

- (You really, absolutely must know which ions are more concentrated on which side of the cell membrane.)

Manipulating the values

4. Start steadily increasing the Na permeability. Note how the RMP changes relative to the amount of change in Na permeability. Where do you think the maximum is?
5. Increase the Na permeability to a very very large number. Confirm the prediction from previous question. Why would this value be the limit?
6. Click the default button at top right to reset to the neuron default values. Now reduce the Cl permeability to 0. What would this correspond to physiologically?
7. Increase the Cl permeability to 120. Note the new RMP. Determine how high the Na permeability needs to be to bring RMP to -45mV.
8. Reset to the neuron default values. Bring the Cl permeability back to 0. Note the new RMP. Determine how high the Na permeability needs to be to bring RMP to -45mV.
9. Cl channels are involved in inhibition of action potentials. Compare the answers to the previous two questions. Explain why the Na permeabilities are different for these two situations even though the RMP was very similar to resting.
10. Reset to the neuron default values. Increase the extracellular K concentration. How does this affect Nernst potential and RMP? Do you think this neuron is more or less easy to depolarize now?
11. Reset to neuron default values. Double the permeabilities of Na, Cl, and K and note the change in RMP. How can this be explained using the Goldman equation for the resting membrane potential? If it doesn't change RMP, how does increasing the number of leak channels change the electrical properties of the neuron?

Action Potentials

Theory

1. The last module covered the status of the axonal membrane at steady-state, where only leak channels were open. There are multiple other kinds of channels in the membrane which can be triggered by various stimuli.
    a. **Ligand-gated sodium channels**. These tend to be found in the dendrites and cell body, and aren't important in conducting the action potential down the axon. However, they are the channels that let sodium into the cell initially, later causing the action potential. Neurotransmitters can open the channels, which then allow Na to flow into the cell with its concentration gradient. (Remember from the last module that Na is more concentrated outside than inside the cell at steady-state.) Other ligand-gated channels exist, like Cl and Ca channels, which influence whether the cell fires.
    b. **Voltage-gated sodium channels**. These channels propagate the action potential.
        i. **Closed state**. Recall that the normal resting membrane potential of a neuron is around **-65mV**. At this voltage, the voltage-gated sodium channels are not open, i.e., sodium permeability stays low.
        ii. **Active state.** When enough ligand-gated channels allow enough Na into the cell for the voltage to increase to about **-45mV**, the voltage-gated channels become active. This increases the permeability of the membrane to Na, bringing its voltage close to the sodium reversal potential, usually around **+40mV**. This in turn increases the voltage of the next segment of membrane, where more voltage-gated sodium channels open, and the increase in voltage **propagates** down the axon.
        iii. **Inactive state**. In order to prevent the action potential from propagating in the wrong **direction**, the voltage-gated sodium channels become inactive after spending some time in the active state. In the inactive state they will not allow sodium to flow even while the voltage is still high (**absolute refractory period**). This allows the other channels to bring the potential back to resting for the next action potential. The inactive channels return to the closed state in a few milliseconds; this delay makes the action potential directional, but limits the rate at which action potentials can be transmitted.
    c. **Voltage-gated potassium channels.** While the voltage-gated sodium channels are very quick to respond to voltage changes, the potassium channels are much slower. They remain closed until the voltage spike has almost reached +40mV, when they start to open. They make the membrane highly permeable to potassium at the same time that the sodium channels are going into their inactive state, dragging the membrane potential back to near the potassium reversal potential. At this point the channels close, but the potential is a little lower than resting (hyperpolarization). The voltage-gated sodium channels have usually returned from the inactive to the closed state by now. They can now be activated, but a stronger stimulus will be necessary to overcome the slight hyperpolarization (**relative refractory period**).
    d. **Sodium-potassium exchanger.** The pump and K leak channels return the voltage to its usual state.

2. This simulation only shows the voltage of a single segment of axon over time.
3. For the purposes of this simulation, only sodium and potassium concentrations and permeabilities are shown in the top left.
4. Note the four controls for stimulus underneath the concentrations. The default values for these will stimulate the axon with a single pulse of 10 mA; however, by changing the number of pulses or the amplitude, you can change the stimulus to multiple pulses.
5. In addition to the voltage of the membrane, the graph shows permeabilities for Na and K over time.

Questions for the Action Potential module

Before you start pressing buttons, make some predictions.

1. Consider the definitions of absolute and refractory periods. Describe a series of experiments that would demonstrate the existence of the refractory periods.
2. What do you think would happen to the action potential if sodium channels were prevented from going into the active state? The inactive state? How about preventing potassium channel opening?

Manipulating the values

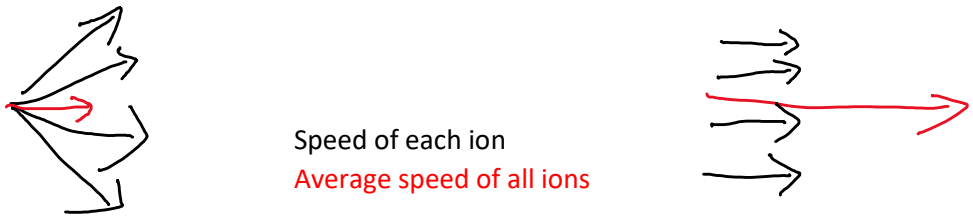
3. Use the default stimuli to stimulate the axon. Note the shape of the action potential, and use the conductance values to determine what channels are open during each segment of the potential.
4. Try lower values of amplitude with 1ms stimulus time until you find a value that does not trigger an action potential. What is the maximum voltage with this stimulus? This voltage is approximately the threshold for triggering the action potential. Does the voltage dip below the resting membrane potential at any point during this stimulus?
5. Keeping the stimulus at the new lower value, increase the amount of time it's applied until you get an action potential. What do you think is the approximate relationship between the amplitude/time combinations needed to stimulate an AP?
6. Drop the stimulus to 5mA, decrease the time back to 1ms, and use 3 pulses with a spacing of 2ms. Note that this does not trigger an action potential. Decrease the spacing to 1ms and note that this has triggered an action potential. Think through how multiple separate stimuli might trigger an action potential when the single stimulus wouldn't, similar to the mechanism in question 5.
7. Use the simulation to demostrate the refractory periods. Try to use the experiment you thought of in question 1 to see if you can reproduce the refractory period phenomena. If you can't, the following stimuli should work:
    a. Two 1ms pulses of 10 mA with spacing 20ms – two normal APs
    b. Two 1ms pulses of 10 mA with spacing 10ms – one normal AP without second AP

    c. Two 1ms pulses of 20 mA with spacing 10ms – two normal APs. This demonstrates the relative refractory period – a very strong stimulus can elicit a second AP, but the original stimulus cannot do it.
    d. Two 1ms pulses of 25mA with spacing 5ms – one normal AP. This demonstrates the absolute refractory period – an absurdly large stimulus cannot trigger an AP until the sodium conductance has come back to normal.
8. Set the stimulus back to two 1ms pulses of 10 mA with spacing 20ms. Stimulate to get an idea of the normal response to this stimulus. Then try it again with each of the three blockers in the drop-down list. Compare your predictions in question 2 with the results.

Action Potential Propagation

Theory

1. There are two basic kinds of axons: myelinated and unmyelinated. Myelinated nerves are wrapped in the cell membranes of either Schwann cells (in the periphery) or oligodendrocytes (in the central nervous system and optic nerve). The extra lipid layers provide extra insulation for the current travelling down the axon, and also cause the membrane to have less channels in it, which makes it less permeable to ions.
2. APs propagate down unmyelinated axons because the ions let in by the voltage-gated channels diffuse to the next segment of axon and trigger an AP there.
3. In myelinated axons, the ions continue all the way to the next **node of Ranvier** before triggering another AP. There is a slow decay of the amount of ions as they continue down the length of the **internode**. So, if the internode is too long, not enough ions will get to the node to trigger the voltage-gated channels, and the action potential will die out. Normal myelinated axons in people have internodes long enough to speed up conduction, but short enough not to drop APs.
4. **Myelinated axons generally conduct faster than unmyelinated axons.** It seems strange intuitively that interrupting the AP would increase the speed, so let's think about that for a bit. The key to understanding this is that speed is much greater in the internodes, which are wrapped up in the myelinating cells.
5. Consider the sodium ions that are fluxing into the cell from an AP at one node of Ranvier. In a cell without myelin, these ions could move back across the membrane instead of continuing axially along the axon. Because many ions would be moving in different directions, the overall speed of ions moving toward the end of the axon would be decreased. (left below)
6. If there is myelin, however, the ions cannot go through the membrane and are mostly forced to go down the axon membrane. This makes their average speed goes up. (right below)



7. The appearance that myelin sheaths give of having APs jumping from one node to the next has given this kind of conduction the name of **saltatory conduction**.
8. Different levels of myelination exist. In general, thicker axons (which are already faster at conduction) have thicker myelin sheaths, which cause less decay of the signal and allow even faster conduction. A-alpha fibers have the thickest axons and myelination, and correspondingly longer internodes; B fibers are the most lightly myelinated; and C fibers are unmyelinated. The types of fibers conduct different kinds of information based on how fast they are. C fibers, for example, conduct pain sensation, which can afford to wait a few seconds, but A-alpha fibers transmit proprioceptive sensation, which changes rapidly and is important to the cerebellum's calculation of balance and posture.

Questions

To run this simulation, set up your stimulus as you did for the previous one, and click Stimulate. Once it's done calculating either click on the Play button or use the time slider to see the voltages across the axon at a particular time. The speeds are actually very slowed down so that you can see the effects across a short segment of axon.

1. Stimulate the axon with the default values, then note the amount of time it takes for the signal to reach the end of the axon. Change the myelination type to a couple of other fiber types (particularly the C fiber) and do the same. What is the relationship of the myelination type to the speed of conduction?
2. Note that the conduction down the C fiber looks just like a normal AP. In general, except for myelinated areas, the propagation of the AP in either time or space looks the same except for a scaling by speed.
3. Try to stimulate the axon with a low-amplitude stimulus, so that there is no action potential. Note that instead of regenerating at the node, the stimulus simply continues to decay.
4. You can also try the other experiments from the normal action potential module; taking the cross section by time instead of space changes the way these experiments look, but shouldn't change the general conclusions of why they work.

Answers to the questions

Nernst and Resting Membrane Potential

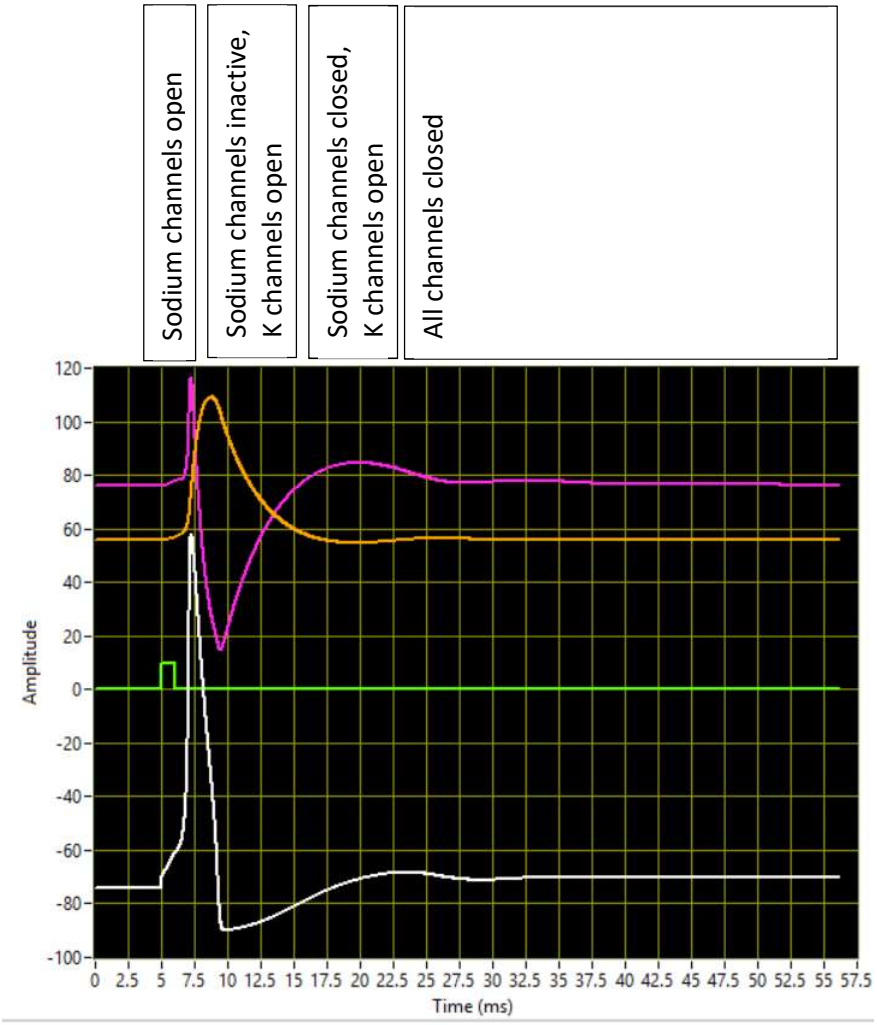
1. K has a very high permeability; Na and Cl have lower permeabilities; Ca permeability is basically 0.
    a) K permeability is due mainly to leak channels; K leak channels are the only leak channels open at rest.
    b) Na permeability is mainly due to Na/K ATPase activity.
    c) Cl permeability is not very important in the axon because the ions move to accommodate the potential created by the Na and K concentrations, but some neurons have Cl pumps that maintain the Cl gradient.
    d) Ca permeability is very low because the ion is fairly large and doubly charged – both impediments to crossing the lipid bilayer. It is important to keep the Ca concentration low in the cytoplasm because Ca is an important modulator and prolonged high [Ca] triggers apoptosis.
    e) Ca has an important role in releasing neurotransmitters at the end of the axon. Voltage-gated calcium channels there allow influx of Ca, which then triggers SNARE proteins to bring neurotransmitter vesicles to the clel membrane.
2. The Na/K ATPase pumps 3 Na ions out for every 2 K ions pumped in. The excess Na are used for other pumps and co-transporters, like the Na/Ca pump.
3. Na is more concentrated on the outside. K is more concentrated on the inside. Cl is more concentrated on the outside (it travels with Na). Ca is more concentrated on the outside.
4. The RMP increases with increased Na permeability. It increases rapidly at first and begins slowing.
5. At a very high Na permeability (think 100000), the RMP comes essentially to 71mV. This is the Nernst (reversal) potential of Na. At such a high permeability, the Na current overwhelms any other ions that try to move the RMP away from the Na Nernst potential where the Na ions are at equilibrium.
6. Setting the Cl permeability to 0 corresponds to closure of Cl leak channels and stoppage of Cl pumps.
7. With Cl permeability = 120, the RMP is -75mV. Increase the Cl permeability to 120. Note the new RMP. Na permeability needs to be around 29 to bring RMP to -45mV.
8. With Cl permeability = 0, the RMP is -69mV. Na permeability needs to be around 15 to bring RMP to -45mV.
9. Question 9 simulated a neuron with Cl channels open, while Question 10 simulated a neuron with Cl channels closed. Na permeability needs to double to bring RMP to the threshold value of -45mV in the high-Cl permeability situation. This is because the Cl reversal potential is close to the resting RMP, and so increasing Cl permeability makes the RMP stay closer to the Cl reversal potential.
10. Increasing the extracellular [K] increases both the K Nernst potential and the RMP. Because it's closer to threshold (-45mV) the neuron should be much easier to depolarize.
11. The RMP should remain the same if the ratio between the different permeabilities is maintained. This is explained by the permeabilities appearing in both the numerator and denominator of the Goldman equation and the increase cancelling each other out. While the

RMP at rest would be the same, any perturbation would be more rapidly corrected because of the high permeabilities. Essentially the open channels “pull” the voltage back towards the resting membrane potential.

12. The RMP and all the Nernst potentials become farther from 0 with an increase in temperature. The reverse happens with a decreased temperature. The Nernst and Goldman equations both directly scale the voltage with the temperature. The temperature has this effect because the rate of ionic diffusion becomes faster with increased temperature.

Action Potential

1. See #7.
2. See #8.
3. See below for the channel states.



4. At a stimulus of 6mA for 1ms, the voltage reaches only about -65mV. Because the voltage-gated potassium channels are activated, though slowly, after the slight increase in voltage, the voltage does dip a little bit before returning to normal.

5. Using a stimulus of 6mA for 2ms, there is still an action potential. Basically the lower the amplitude, the longer the stimulus needed, although there is a threshold below which the current input is simply too weak to overcome the cell's own maintenance of its electrical gradient. The longer stimulus allows more positive charge to flow into the cell even though it's going in slower.
6. Similar to stimulating for longer, multiple stimuli sufficiently close together can get enough positive charge into the cell to trigger the voltage-gated sodium channels.
7. One other thing to note here: even the absurdly large stimulus triggers the exact same AP as the smaller ones. It may take a little less time to develop, but not much in the scheme of things. The action potential spike is always the same height and width, barring changes in sodium, potassium, and chloride concentrations which would change the reversal potentials or resting membrane potential.
8. The active Na blocker should simply prevent an action potential; the inactive Na blocker would allow the first AP, but prevent the second one from firing; and the K channel blocker would prevent the first AP from repolarizing and so prevent the second AP from firing as well. A number of drugs exist which can block these channels in these configurations, but their effects are not quite so drastic – this simulates an irreversible, complete blockage. All drugs have a non-perfect efficacy and almost all are reversible. So, a drug that blocked inactive Na channels would prevent APs from firing, but not forever.

Spatial Action Potential

1. The A-alpha fiber takes the shortest amount of time to conduct the signal to its end, increasing steadily for each fiber type until the C fiber, which is pretty slow.