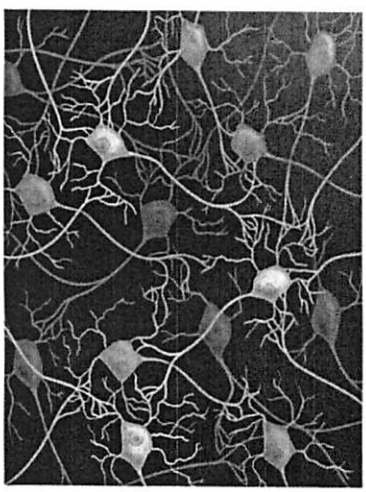
Lab 8: What affects the distance over which an electrical signal is transmitted and the speed of transmission? Testing Models of Signal Transmission Along Nerve Axons.[1]

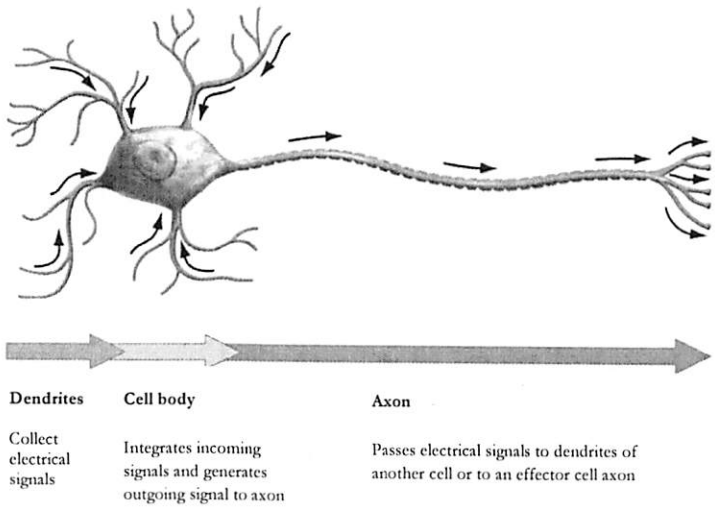
Introduction:

(Note: This two week lab sequence is a little different in structure from labs we have done in the past. We will be focusing explicitly on building and testing models. To prepare you for modeling a phenomenon using ideas we are just starting to cover in class, this lab requires a little more structure and guidance than prior labs have used. Working in lab groups, read through this material and design and test carefully. The **lab write-up** will include (a) your explanations of the models you develop, (b) the supported conclusions you draw from testing and analyses of your models, and (c) your discussion of advanced topics related to the basic ideas you are exploring. **When you see a □, check with your TA/LA** to make sure your overall goal is accomplished.)

Nerve impulses travel in our bodies as electrical signals. Whether it's seeing or hearing something, controlling a muscle, or just thinking, the transmission process along a nerve cell, or neuron, is the same: a sufficient stimulus received by the cell body (*soma*) initiates a change in the potential difference across the membrane, or *action potential*, which travels along the *axon* to be transferred through a synapse to other neurons or muscle cells. [See Figs 1 & 2 below.]



(Fig 1) Neurons form networks for information flow



(Fig 2) Information flow through neuron's axon

A single axon can be a meter or more long, like those connecting our toes to our spinal cord, so action potentials must travel a long way. You may have learned in a biology course that for an action potential to be transmitted from one end of an axon to another, it must be regenerated repeatedly along its length. We call this *active transport*[2] and it is accomplished by voltage-gated ion channels, as discussed in the appendix to this lab. Why is active transport necessary? Couldn't we simply apply a potential difference to the end of a

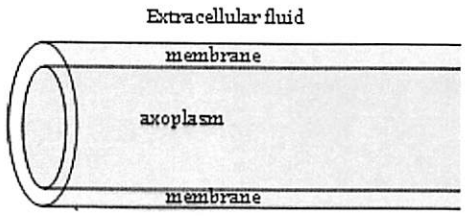
[1] Based on a lab developed by Eric Anderson and Lili Cui, University of Maryland, Baltimore County and adapted by Catherine Crouch, Swarthmore College. Currently adapted with permission by Kim Moore, John Giannini, and Wolfgang Losert (UMd PERG and Biophysics).
[2] The use of "active transport" here is different from our previous use of the phrase when discussing molecular motors (kinesin "walking"). Here we mean transport of a signal by active regeneration. Similarly, "passive transport" is a transport of a signal by passive initial generation with no additional regeneration.

nerve axon and expect that signal to travel along the axon to its final destination, the spinal cord and then the brain? This alternate type of signal transmission is called *passive transport.* In this two week lab sequence, we will be **modeling passive transport** in nerve axons and **testing our model** to determine if passive transport is a feasible method of signal transmission. We will also be **considering some adaptations** to the simple cylinder model of a nerve axon (see below) that can increase the speed of signal transfer—surely a faster response is advantageous to survival in a species!

Simple cylinder model of a nerve axon: Structure and Properties

The structure of the axon is shown schematically in Figure 3 below. For electrical purposes, an axon is basically a long, thin cylinder of membrane filled with a fluid called *axoplasm.* When no action potential is traveling, the potential difference across the membrane, from the outside of the axon to the inside, is about -70 mV, the *resting* potential difference. In part, this is because the concentration of sodium ions (Na^+) is much higher outside the axon than inside.

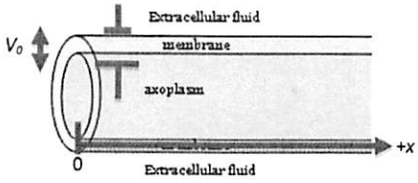
The axoplasm, like all fluids in biological systems, has mobile ions dissolved in it, giving it moderate conductivity[3] (many orders of magnitude lower than copper or other metals we can think of as ideal conductors). The fluid outside the membrane, the *extracellular fluid,* is very similar to the axoplasm and thus has approximately the same conductivity.



(Fig 3) Axon: Simple cylinder model

The lipid bilayer forming the membrane is electrically insulating, with extremely low conductivity, but many different kinds of channel proteins cross the membrane. These channels only allow specific ions to pass under particular conditions. The channels increase the conductivity of the membrane as a whole, so that although the membrane conductivity is much lower than that of the axoplasm, current does pass through the membrane.

Part 1: Modeling Passive Transport



Our goal is to understand how a potential difference applied across the membrane at one end of the axon spreads along the axon if it is not being regenerated as it travels down the axon. Thus for the purposes of our model we'll assume there is a battery at the left end of the axon segment (see the figure), holding the potential difference from the outside to the inside at $x = 0$ to a *positive* value we'll call V_0.

Suppose there was just a single ion channel[4] (e.g., a "potassium leak channel") crossing through the membrane, at the right end of the segment in the figure. **Draw a loop** onto the diagram showing the path of current flow from the positive side of the battery (inside) to the negative side of the battery (outside), and

[3] Recall that conductivity is inversely related to resistivity, ρ. Copper has high conductivity and low resistivity. Axoplasm has low conductivity and high resistivity.

[4] In reality, the current can flow both direction across the membrane (into and out of the axon) through a single "channel"--this can be thought of as a combination of Na^+ and K^+ voltage-gated channels. This is the case for *active* transport. Since we are only modeling ***passive*** transport, we can think of this as a single K^+ ion channel.

then **draw a simple circuit** that corresponds to this pattern of current flow. The axoplasm, an ion channel, and the extracellular fluid should each be represented separately in your simple circuit.

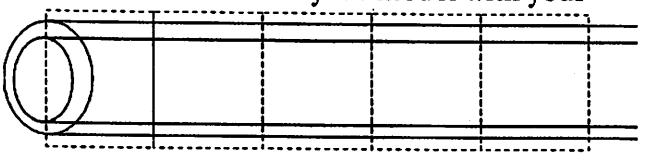
In fact, the resistance of the current path through the extracellular fluid is much less than the resistance of either the axoplasm or the ion channel through the membrane, so the extracellular fluid can be represented as just a conducting wire in the simple circuit. (If you didn't initially do so, update your simple circuit accordingly.) Let's think about why the extracellular fluid will have such low resistance.

Resistance R can be determined from the length L, cross-sectional area A, and resistivity ϱ according to $R = \frac{\rho L}{A}$. The resistivities of the axoplasm and the extracellular fluid are very similar.

? Considering how the current flows through both the axoplasm and the extracellular fluid, **explain why** the resistance of the current path through the extracellular fluid is much smaller than the resistance of the current path through the axoplasm—thus we can treat the extracellular fluid as just a conducting wire.

A long axon can be modeled as a chain of many short segments like the segment described above. The dashed lines in Figure 4 below visually divide the axon into five such segments. Sketch the path of

! current flow onto the diagram and then **design a circuit model** for it. **Check your model with your instructor.**



(Fig 4) Axon: chain of many segments

To complete the model, we need values for the resistances. There are actually many ion channels passing through the membrane, so our model circuit needs to be constructed using the resistance of a segment of membrane rather than a single ion channel. Each segment of membrane consists of many ion channels in parallel, so the overall resistance of the membrane segment is the equivalent resistance of all of those ion channels. The average resistivity of the membrane can be measured and used to find the resistance of a segment of membrane.

! Use the relationship between resistance, resistivity, length, and cross-sectional area to **estimate values for the resistances** of a membrane segment R_{mem} and an axoplasm segment $R_{axon,}$ using the following order-of-magnitude values:

- the diameter of the axon ~ 10 μm
- the membrane thickness ~10 nm
- the resistivity of the axoplasm ~ 1 Ω-m
- the average resistivity of the membrane ~ 10^8 Ω-m
- the segment length ~ 1 mm

Hint: To determine the cross-sectional area of a membrane segment, think of "unrolling" the membrane from the axon. Also, it turns out that only the ratio R_{mem} /R_{axon} affects how far the potential difference will spread.

□ *Part 1, Overall Goal:* **Design circuit model with appropriate resistances.**

?= Some questions to help guide your work.

!= A question/problem to which you must EXPLICITLY respond in your write-up.

Part 2: Qualitative Analysis of Your Model

With the model you have created, we hope to understand how the potential difference across the membrane changes as we examine different distances along the nerve axon. In other words, how is the voltage[5] across the membrane at a distance x related to the initial voltage, V_0? Before we collect data and perform a quantitative analysis of your model, let us **think qualitatively about some of the features of your model of passive transport.**

We want to understand how the voltage across the membrane depends on the distance along the axon. Each segment in your multi-segment model circuit should include a resistor representing the membrane, so the voltages across these resistors represent V_{mem} at each segment. The first R_{mem} corresponds to segment 1, the second to segment 2, and so on. Let's notate these voltages as V_{mem}^{1}, V_{mem}^{2}, and so on. Similarly let's call the voltages across the axon segments V_{axon}^{1}, V_{axon}^{2}, and so on; the currents in the axon segments are I_{axon}^{1}, I_{axon}^{2}, and so on.

? **Applying the loop rule**[6] to the first segment, find V_{mem}^{1} in terms of the applied membrane potential V_0, the current in the first axon segment I_{axon}^{1}, and the resistance R_{axon}. Do the same for the second segment, finding V_{mem}^{2} in terms of the applied membrane potential V_0, the axon currents, and the resistance R_{axon}. Is V_{mem}^{2} less than, equal to, or greater than V_{mem}^{1}? How do you know? Do the same for the third segment, finding V_{mem}^{3} in terms of the applied membrane potential V_0, the currents in the segments, and the resistance R_{axon}. With *n* indicating the segment number, does V_{mem}^{n} increase or decrease as *n* increases? Does ! I_{axon}^{n} increase or decrease as *n* increases? On your circuit diagram, draw arrows of different widths (or lengths) by each axon resistor to indicate the amount of current in that axon segment.

From your analysis, it should be clear that V_{mem} decreases as you go further and further from the start of the axon. Can we be more specific? If we create a mathematical model for $V_{mem}(x)$ as a function of distance x from the start of the axon, what functional form would we expect? As a reminder, common possibilities include: (1) linear with a negative slope (rate of change is constant), (2) inverse (product is constant), and (3) exponential decay (percent change is constant). It should help to consider the behavior of $V_{mem}(x)$ in the limiting cases. Examine the behavior of your model circuit. As x approaches 0, what ! happens to $V_{mem}(x)$? As x becomes very large, what happens to $V_{mem}(x)$? Compare with the behavior of each of the possible functional forms. Make your best guess for the functional form and sketch the corresponding graph of $V_{mem}(x)$ vs. x. Explain your reasoning.

□*Part 2, Overall Goal:* **Describe qualitative features of the model circuit in both equation and graphical forms.**

?= Some questions to help guide your work.

!= A question/problem to which you must EXPLICITLY respond in your write-up.

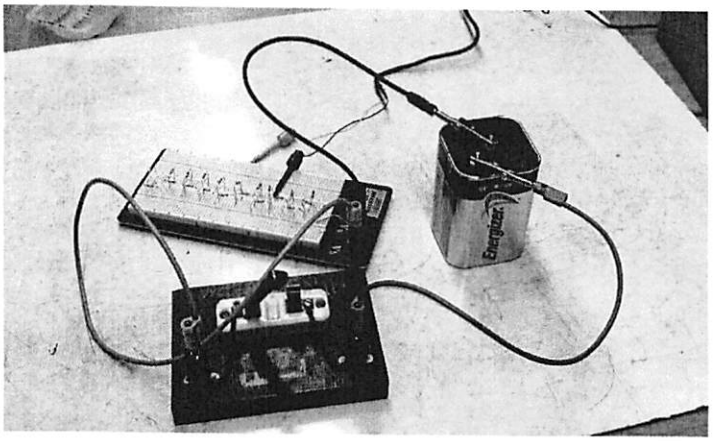
[5] "Potential difference" and "voltage difference" are synonymous terms. "Potential difference" is useful because it highlights the connection to the idea of "action potential," although an action potential is a particular way that potential differences change over time along the axon. For the remainder of this lab we will use "voltage" instead of "potential difference" because it is a shorter word.

[6] The Kirchhoff Loop Rule says that the sum of voltage changes across each element in a loop is zero—i.e., all that you gain is eventually lost.

Part 3: Quantitative Analysis of Your Model

We are now ready to build and test your passive transport circuit model.

Equipment:

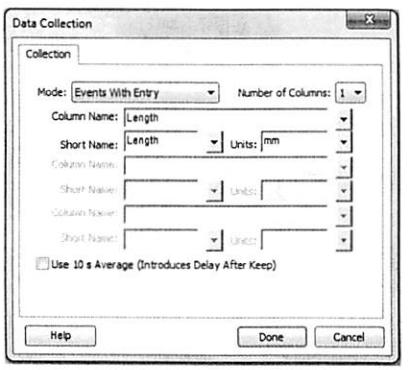


- circuit boards, 2
- connecting wires, 3
- alligator clips, 2
- battery
- switch
- assorted resistors (10 each of three different kinds)
- voltage probe
- LabPro
- computer and LoggerPro software

The circuit board (also called a 'bread board') is probably new to you and requires a short explanation. Down the center of the board are two columns of holes surrounded by a red and a blue line. Leave the negative end of the battery connected to the blue line; this is your extracellular fluid and all of these holes are connected together in this column (connected inside the circuit board). The other parts of the circuit board are much wider sets of columns. For these, the holes in a column are NOT connected, but the holes in a ROW ARE. If this is not clear enough for you, ask a TA or LA to explain. You will need to think carefully about how you can fit your model of passive transport in a nerve axon onto this circuit board. You have two circuit boards so that you can begin building a second model with spare resistors once you are ready to test the first model.

Construct your model circuit with ten segments using the provided components. Choose resistors with the proper ratio of resistance values that you found in the previous part, and make sure that you use the same value of resistance for all of the R_{mem} and a different constant value for all of the R_{axon}. Don't forget to close the switch to take data and open it when you finish.

Measure and record V_{mem}(x) vs. x for x=0 mm to x=10 mm. (In your circuit, each axon resistor corresponds to a 1 mm segment, so for your measurements, x is the segment number. At x=0, V_{mem}(x) = V_0.) To record voltages in LoggerPro:



- Click on the Data Collection icon  , and change the mode to "Events with Entry." Provide appropriate name and units. Click Done.
- Press the start button.
- Use the voltage probe to measure voltages by attaching the probe leads to the circuit on either side of each R_{mem}. To store voltage values in a table, click on "Keep Current Value". You can enter the corresponding length value in the same row of the table. Don't

forget to collect the x=0 voltage value, V_0!

- If a Data Erase box appears, click on Append to Latest Data and proceed.
- Press the stop button when finished with all 11 data points.
- To display data points without connecting lines, double click on the graphed data, and unselect the option for connecting the data points.

Now let's **interpret your results.** Following the same line of reasoning you used in your qualitative analysis to find V_{mem}", and assuming that the segment is only a differential length *dx* long, it is possible to derive a differential equation for $V_{mem}(x)$—don't do this, just know that it is possible! Solving the differential equation gives us an equation describing how the voltage across the membrane depends on distance x from the end where a voltage V_0 is applied. The equation is then:

$$V_{mem}(x) = V_0 e^{-x/\lambda} \text{ with } \lambda = \alpha\sqrt{\frac{R_{mem}}{R_{axon}}};$$

where λ (Greek letter lambda, called the *length constant* of the axon) is the distance along the axon at which $V_{mem}(x)$ has decreased by a factor of *e* (the natural logarithm base e), to $0.37V_0$; and α is a proportionality constant with units of length that is specific to different types of axons.

To perform a fit:

- Click "Analyze"
- From the drop down menu select "curve fit"
- From the pop-up menu select the appropriate function for exponential decay
- Click "Try Fit"
- If it is the correct fit click "Ok"

! Provide the graph of your data with your best fit function in your lab write-up. Calculate the length constant of your model circuit from your best fit function. What does this length constant tell you about the feasibility of passive transport as a signal transmission method? Will your brain ever be informed of an injury to your toe? Given the length constant you have determined, how many 'lengths' are there in the nerve connecting your toe to the base of your spine? What fraction of the original potential difference (V_0 at your toe) remains by the time the signal reaches the spine?

Build two new model circuits with two different length constants and find these length constants with a few measurements (you don't have to measure as many data points if you choose them wisely; include the graphs for these two circuits in your report). (***Hint:*** You have three different sets of resistors. How many ways can you pair them up to build a circuit? What ways have you already used?) How does the

! length constant change as the ratio of R_{mem} to R_{axon} changes? To get a signal from your toe to reach the base of your spine using passive transport, **what would the ratio of resistances need to be?** State your assumptions.

□ *Part 3, Overall Goals:* **Determine over what lengths passive transport is effective. Calculate the length constant for a specific R_{mem}/R_{axon} and determine how λ changes when the resistance ratio changes.**

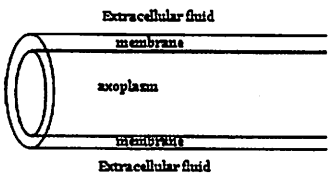
? = Some questions to help guide your work.

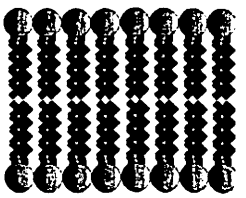
! = A question/problem to which you must EXPLICITLY respond in your write-up.

Part 4: What adaptations allow the action potential to travel faster?

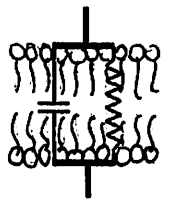
How fast does an action potential travel along an axon? You're probably aware that it's finite—no one has truly instant reflexes. Yet, your model circuit suggests that the process is nearly instantaneous. Something is missing from this model. We need to extend our model in order to understand *why* it takes time for an action potential to travel along an axon.

Let's take a closer look at the membrane of an axon. [See Fig 5 below.] The channels are the paths through which ions flow (with some resistance). The equivalent resistance of all of these parallel channels in a single segment is R_{mem}. Now take away those channels and what do you have left? An insulating layer with conducting fluids on each side. That should remind you of a familiar device—a capacitor. The membrane is therefore properly viewed as a resistor and capacitor in parallel. [See Fig 6 below.] As current travels along the axon and enters each new segment, charge also accumulates on the capacitor. It takes a significant amount of time for a capacitor to charge and thus also for the potential difference across the membrane to reach its final value. We refer to the time it takes the membrane potential difference to reach 63% of its final value as the time constant τ. It can be shown that $\tau = R_{mem}C_{mem}$, where R_{mem} is the resistance and C_{mem} is the capacitance of a segment of membrane. It takes several time constants for the membrane potential differences to reach the values that your model resistor circuit gives.





(Fig 5) Membrane: magnifying view



(Fig 6) Membrane: RC circuit model

Speed depends on time constant and length constant

The speed at which an action potential travels down an axon depends primarily on two things:

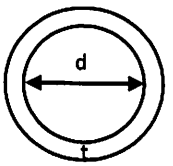
• **It's *inversely proportional* to the time constant**: The longer it takes the membrane potential difference to rise in each segment, the slower the action potential will travel. The time constant is given by $\tau = R_{mem}C_{mem}$.

• **It's *proportional* to the length constant**: The greater the length constant, the farther the depolarizing potential difference reaches down the axon without regeneration, bringing successive segments to the threshold potential difference required to regenerate the action potential sooner. You measured the length constant of your model circuit in Part 3. In general, the length constant λ is proportional to $\sqrt{\frac{R_{mem}}{R_{axon}}}$. This makes sense: If the membrane resistance is really big (or if the axon resistance is really small), current mostly flows *down* the axon, with just a little flowing across the membrane in each successive segment.

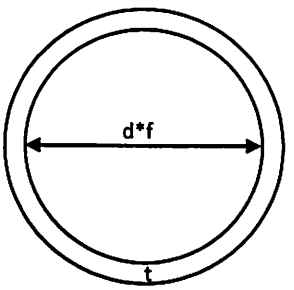
It makes sense that faster propagation of nerve impulses confers an advantage to an organism. In the next few questions, we'll **examine the physics behind some adaptations leading to speedier action potentials.**

! **A: How does making the axon wider (larger diameter) affect the speed?**

Let's say the diameter of the axon is increased by a factor of *f*. [Fig 7]



(Fig 7) A wider axon



1. By what factor does R_{axon} change? (Note: The resistivity of the axoplasm is constant, as is the 1 mm length of an axon segment.)

2. By what factor does R_{mem} change? (Note: The resistivity of the membrane is constant, as is the 1-nm thickness of the membrane.)

3. By what factor does the length constant λ change?

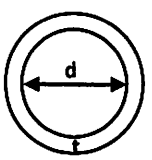
! 4. Assuming that the time constant doesn't change, by what factor does the speed therefore change?

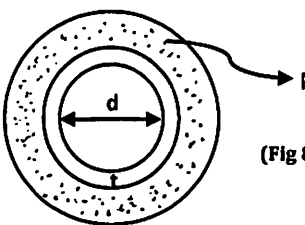
! 5. By what factor would the diameter of the axon have to change to increase the speed by a factor of 10?

Note: The 'wider axon adaptation' is the strategy adopted by the squid, whose "giant" axons allow very rapid travel of its action potentials, making it a master of the quick escape.

! **B. What about making the membrane thicker?**

Wider axons work fine for a squid, but are highly impractical for organisms with lots of neurons like humans. (If each of your neurons were the size of a squid's, your head wouldn't fit through a doorway!) Let's explore another possible way of increasing the length constant and therefore the speed: increasing R_{mem}. This is the strategy commonly adopted by vertebrates. It's achieved by extra insulation (a myelin sheath) that's wrapped around the axon. [Fig 8]





(Fig 8) A myelinated axon

? 1. Let's say that myelination increases the membrane resistance by a factor of 1000. By what factor does the length constant increase? Why?

! 2. Assuming that the time constant doesn't change, by what factor does the speed therefore change? Why?

! 3. Based on the analysis you did in Part 3, could a myelinated axon use passive transport as the signal transmission method? Why or why not?

4. There are many diseases that affect signal transmission along neural pathways. These include multiple sclerosis (MS), myasthenia gravis, and amyotrophic lateral sclerosis (ALS or 'Lou Gehrig's Disease'). Often, a component of these afflictions is a malformation or degeneration of the myelin sheath. MS is a demyelinating disease, which means that the axons of neurons are intact but the myelin sheaths are damaged. Why would loss or damage to the myelin sheath be a problem for signal transmission, even if the axon was intact?

! Note: Myelination obstructs the membrane's voltage-gated Na^+ channels that enable the action potential to be regenerated. For this reason, there are gaps in the myelin coating, called the nodes of Ranvier, where the channels are not obstructed. From what you've learned in this lab, what do you suppose determines the maximum distance between these gaps?

□ *Part 4, Overall Goals:* **Determine the effect of adaptations (wider axon, myelination) on signal speed along nerve axon. Consider complications caused by myelination defects.**

For your report:

As stated previously, the **lab write-up** should include (a) your explanations of the models you made, (b) the supported conclusions you drew from analyses of your models, and (c) your discussion of advanced topics related to the basic ideas you are exploring. To be more specific, you should discuss the work you have done and the considerations you have made in modeling passive transport, qualitatively and quantitatively analyzing your model, and modeling adaptations that can increase the speed of signal transfer. If you want to include hand-drawn elements (such as model circuit diagrams), please do so. Don't forget to include the LoggerPro plots of $V_{mem}(x)$ vs. x with the appropriate fitting functions. Respond explicitly to all passages marked with an exclamation point (!).

Approximate timing:

Week 1:

- Introduction ..... 15 min.
- Modeling (Part 1) ..... 40 min.
- Qualitative Analysis (Part 2) ..... 30 min.
- Quantitative Analysis, Circuit 1 (Part 3) ... 25 min.

Week 2:

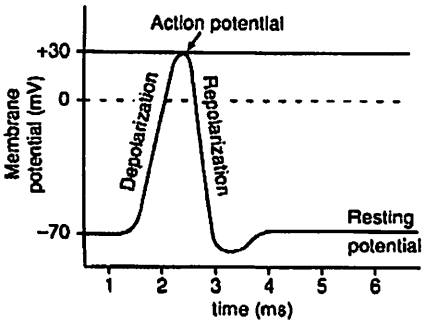
- Finish Quantitative Analysis (Part 3) ..... 35 min.
- Adaptations for Speed (Part 4) ..... 45 min.
- Finalize Report ..... 20 min.
- Class Discussion/Presentations ..... 10 min.

?= Some questions to help guide your work.

!= A question/problem to which you must EXPLICITLY respond in your write-up.

Appendix: Brief summary of action potential regeneration by voltage-gated ion channels

The basic idea of the action potential is as follows. The neuron's cell body combines incoming electrical signals from different stimuli and sends the combined signal to the axon. If that signal increases the membrane's resting potential difference by more than about 20 mV (from -70 mV to a *threshold* of about -50 mV), then *voltage-gated Na^+ channels* in the axon membrane immediately adjacent to the cell body open and allow Na^+ ions to flow into the axon. This launches the action potential (see figure at right).



Driven by both the concentration gradient and potential gradient, Na^+ ions pour in until the membrane potential difference changes from -70 mV to about +30 mV, a process referred to as *depolarization*. Because the depolarized region into which Na^+ ions have flowed is at positive potential compared to the rest of the axon, positive ions flow away from it, along the axon. Some of the current continues along the axon, while some travels through the membrane into the extracellular fluid and back to the negative region outside the axon at the site of the beginning of the action potential. As positive ions flow along the axon, the membrane potential difference in neighboring regions becomes less negative. As the threshold potential difference is reached, voltage-gated Na^+ channels in the membrane open, initiating a new depolarization, which regenerates the action potential. A millisecond or so after the start of the depolarization in each region, the voltage-gated Na^+ channels close, and the resting potential is re-established. In this way, a change in membrane potential difference travels undiminished along the axon.