# Lab 6: Examining the Passive Spread and Speed of Nerve Signals[1]
## Swarthmore College Introductory Physics for the Life Sciences

**Objectives:**
> To design and make measurements on a resistor model for the **passive** spread of an electrical stimulus applied to one end of an axon.
> To understand why an action potential applied at one end of an axon must be continually regenerated along its length.
> To describe the factors that affect the speed of propagation of an action potential, and understand how changes in axon diameter and myelination affect speed.

**Preparation:**
Read over the lab writeup, write a paragraph summarizing the purpose of the lab, and give very brief answers to the following questions (no explanation needed for 1 or 3):
1. Is a resistor made of conducting material or insulating material?
2. What distinguishes a resistor from the wires that connect the components of a circuit?
3. What is the relationship between resistance and conductivity?

**Introduction**
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 strong enough stimulus received by the cell body (*soma*) launches a change in the potential difference across the membrane, which travels unchanged along the *axon*. Such a traveling signal is referred to as an *action potential*.
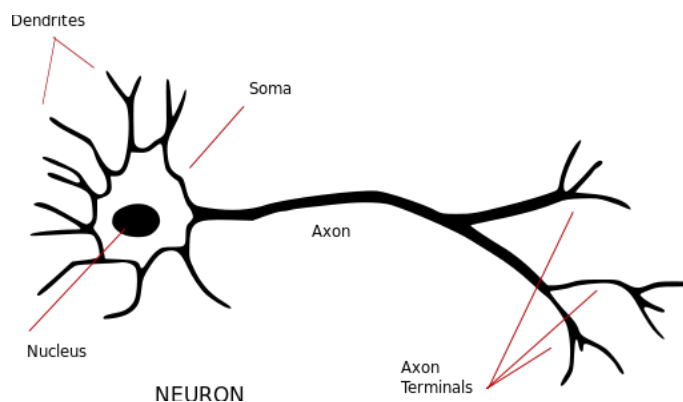


Figure 1. Schematic showing the parts of a neuron. Figure from Wikimedia Commons.

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A single axon can be a meter or longer, such as 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; this is accomplished by voltage-gated ion channels, as discussed in the appendix to the lab.

In this lab, you will examine two questions:

1. **Why is it necessary to keep actively regenerating the action potential?**
2. **What factors affect the speed at which a signal can travel along an axon**?

To answer the first question, you and your lab partner will build a circuit model of an axon that lacks voltage-gated ion channels, and thus lacks the ability to actively regenerate changes in membrane potential. You will measure how the potential difference across the membrane changes with distance from the end at which a signal is applied. To answer the second, you and your lab partner will reason about the effects of changing axon size or membrane thickness.

## Potential difference, membrane potential, and action potentials

To answer question 1 clearly requires being specific about what an action potential is. Being precise with language and notation will facilitate this, so we begin with a short set of definitions and the notation that goes with them.

In cell biology and electrophysiology, the term "membrane potential" typically refers to the potential difference across the membrane. The term "membrane potential" makes sense in the context of the specific ways this is typically measured.

An instrument that measures potential difference has two electrodes, and detects the potential difference between them. In last week's lab, when measuring voltages in a circuit, you probably moved both electrodes around to make numerous different measurements. However, in an electrophysiology experiment, the two electrodes are typically kept in fixed locations. The positive (measurement) electrode is placed inside the cell, and the negative (reference) electrode is placed outside. The instrument thus measures the potential difference across the membrane going from outside to inside. To make all this explicit, in this course we have notated the potential difference across the membrane as follows:

$$\Delta V_{membrane} = V_{in} - V_{out}$$

The $\Delta$ in $\Delta V_{membrane}$ makes it clear that this quantity is a difference measured between two locations.

There is an alternate way to think about this measurement. As with potential energy, we are always free to choose the location where $V = 0$. When making a measurement, the location of the reference electrode is conventionally the place chosen for $V = 0$. This is the way of thinking that

informs the term "membrane potential". The outside of the cell is chosen to be the location where $V = 0$, and then the potential of the inside, which we've called $V_{in}$, is called the "membrane potential" and often notated $V_m$.

Because the term "membrane potential" and the notation $V_m$ are the most common approach in neuroscience, we will use them for the rest of this lab. **As you work, keep in mind that $V_m$ and what we've been calling $\Delta V_{membrane}$ are different ways of notating exactly the same quantity.** Writing $V_m$ does not call attention to the choice of reference location, so you have to remember that such a reference location is required, and for the "membrane potential," always chosen to be the outside of the cell.

The resting membrane potential for any kind of cell is negative, because the charge layer on the inner surface of the membrane is negative and that on the outer surface is positive. The exact value varies between types of cells, but is typically –70 mV to –90 mV.

An action potential is a localized *change* in $V_m$ from the normal resting value to a positive value (corresponding to a membrane with the charge distribution more positive on the inside than the outside) which is initiated at one end of the axon, and then travels unchanged down the entire axon. "Travels unchanged" means that as the action potential passes through, each bit of the membrane reaches the *same* positive value (and then returns to resting value). So, for example, if the action potential has a peak value of $V_m = +30\text{mV}$ as in the Appendix, every place along the axon reaches that potential briefly when the action potential reaches it, and then drops back to resting potential after the action potential has passed through.

## Experiment I: How far can a potential difference passively spread along an axon?

Question 1 asked why active regeneration of the action potential is necessary. To answer this question experimentally, we will use a model circuit to determine how far a change in membrane potential spreads *without* active regeneration.

### A. Designing an electric circuit to model an axon

In order to design a model circuit, let's begin by thinking carefully about how the properties and behavior of the axon can be modeled using resistors and batteries. This lab will step you through the process of designing the circuit and making measurements on it.

### The axon: Structure and properties

The structure of the axon is shown schematically in Figure 2. For electrical purposes, an axon is basically a long, thin cylinder of membrane filled with a fluid called *axoplasm* (or *cytoplasm*, which is the term for the fluid inside any kind of cell).

The axoplasm, like all fluids in biological systems, has mobile ions dissolved in it, giving it moderate conductivity (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 has approximately the same conductivity.
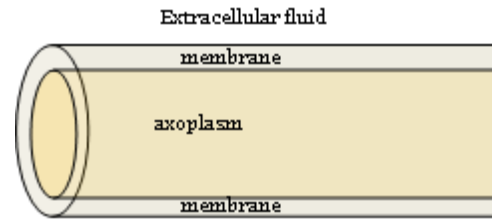


Figure 2. Simplified axon geometry.

The lipid bilayer forming the membrane is electrically insulating, with extremely low conductivity, but many different kinds of proteins cross the membrane. These proteins allow specific ions to pass under particular conditions, and 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. Even when the voltage-gated ion channels are closed, the other proteins give the membranes a modest conductance.

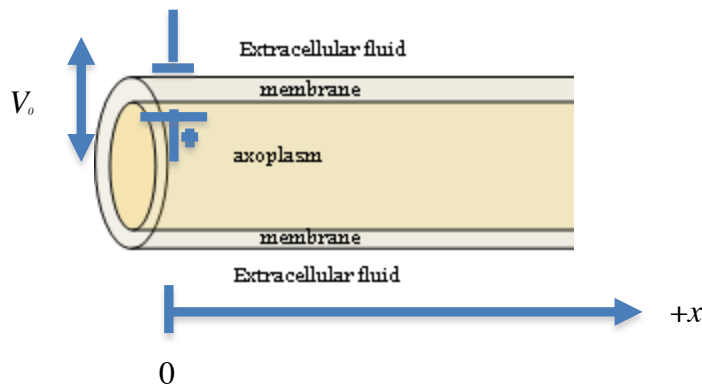**Designing the model circuit**



Figure 3. Applying an electrical signal to the axon.

Our goal is to understand how a change in $V_m$ applied at one end of the axon spreads along the axon if it is not being regenerated as it travels down the axon. We can model this by thinking of attaching a battery at the left end of the axon in the figure above, holding the potential of the inside at $x = 0$ to a *positive* value we'll call $V_0$. (Recall that the resting membrane potential normally negative, meaning that the inside is negative compared to the outside.)

Work with your lab partner to answer the following questions:

1. Start by considering the simplest possible model. Suppose there was just a single place that current could cross the membrane, at the right end of the segment. **Draw a loop onto Fig. 3 showing the path followed by the current from the positive side of the battery (inside) to the negative side of the battery (outside). Then design a circuit that matches this pattern**

**of current flow and sketch it in your lab notebook. Label each circuit component with a symbol that makes it clear what part of the neuron it represents (for example: if you represent the axoplasm as a resistor you could label that resistor $R_{ax}$).** The axoplasm, the membrane, and the extracellular fluid should each be represented in your model circuit.

2.  In fact, the resistance of the current path through the extracellular fluid is much less than the resistance of either the axoplasm or the membrane, so the extracellular fluid of each segment can be represented as just a conducting wire in the circuit. If you didn't initially do so, update your circuit from #1 accordingly. The rest of this question should help you understand why.

    The resistance $R$ of a conductor can be determined from its length $L$, cross-sectional area $A$, and conductivity $\sigma$, according to $R = \dfrac{L}{\sigma A}$. The conductivities of the axoplasm and the extracellular fluid are very similar. **Explain why the resistance of the current path through the extracellular fluid is much less than the resistance of the current path through the axoplasm, so we can model the extracellular fluid as a conducting wire.** Ask your instructor if you get stuck.

3.  Now let's consider a multi-segment axon, as shown in Fig. 4; the dashed lines divide the axon into a chain of segments. Suppose current can cross the membrane at the right end of each segment. Sketch the path of current flow onto the copy provided of this diagram (so that you can put it into your lab notebook) and then design a circuit model for it. **Check your model with your instructor and draw the final model circuit in your lab notebook. Be sure to label your circuit with the appropriate resistances ($R_{ax}$ or $R_{mem}$).**
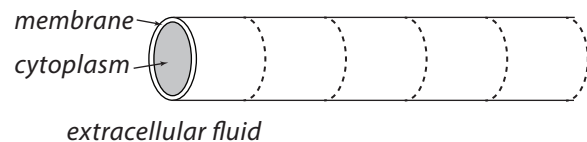


Figure 4. Multi-segment axon.

In fact, ion channels that allow current to pass through the membrane are spread over the entire membrane, not just at a single place in each segment, so in our model circuit, each resistor $R_{mem}$ represents the equivalent resistance of a segment of membrane. The average conductivity of the membrane can be measured, and then this value can be used to find the resistance of a segment of membrane.[2]

4.  To complete and build the model, we need values for the resistances. Let's consider a segment 1 mm long.[3] **Use the relationship between resistance, conductivity, length, and**

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[2] Each ion channel offers a parallel path for current, so the equivalent resistance of the membrane segment is the equivalent resistance of all its ion channels in parallel.

[3] The length of the segment is completely arbitrary. Choosing a segment 1 mm long gives resistances that are easy to work with in the laboratory. As you'll see, the conclusions we draw from this lab don't depend on the choice of the segment length.

**cross-sectional area to estimate values for the resistances of a membrane segment, $R_{mem}$, and an axoplasm segment, $R_{ax}$, using the following order-of-magnitude values:**

- axon diameter ~ 10 μm
- membrane thickness ~10 nm,
- axoplasm conductivity ~ 1 A/(V•m)
- average membrane conductivity ~ $10^{-8}$ A/(V•m)

Hint: To determine the cross-sectional area in order to calculate the resistance of a membrane segment, think of "unrolling" the axon.
**It turns out that only the ratio $R_{mem}$ /$R_{ax}$ affects how far the potential difference will spread. Record this ratio in your lab book.**

5. You might be wondering why this circuit has resistors and batteries, but no capacitor. It turns out that although the membrane capacitance affects the *speed* with which the change in membrane potential spreads, just as in a RC circuit the presence of the capacitor affects the time dependence of the current and voltage, the capacitance doesn't affect the *distance* it spreads. We will consider the membrane capacitance when we get to answering question 2!

**B. Qualitative analysis of your model circuit.**

We want to understand how the voltage (potential difference) across the membrane depends on the distance along the axon. Each segment in your model circuit should include a resistor representing the membrane, so the voltages across these resistors (measured from outside to inside!) represent $V_m$ 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 $V_m(x=1)$, $V_m (x=2)$, and so on, in which the units of $x$ are the length of a single segment. Similarly let's notate the currents in the axon segments $I_{axon}(x=1)$, $I_{axon}(x=2)$, and so on.

(1) Does $I_{axon}(x = n)$ increase or decrease as $n$ increases? How do you know?
On your circuit diagram, draw arrows of different sizes by each axon resistor, with the size indicating the amount of current in that axon segment.

(2) Applying the loop rule to the first segment, find $V_m(x = 1)$ in terms of the excitation $V_0$, the current in the first axon segment $I_{axon}(x = 1)$, and its resistance $R_{axon}$.

(3) Do the same for the second segment: find $V_m(x = 2)$ in terms of $V_0$, the resistance $R_{axon}$, and the currents $I_{axon}(x = 1)$ and $I_{axon}(x = 2)$.[4] Is $V_m(x = 2)$ less than, equal to, or greater than $V_m(x = 1)$? How do you know?

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[4] Eliminating the currents from this expression can be done but involves some clever mathematics. Your lab instructor can show you how if you are curious; the purpose of these questions is to figure out what happens to $I_{axon}$ and $\Delta V_{mem}$ as the distance from the applied potential increases.

By Catherine Crouch and Benjamin Geller, Swarthmore College, 6/2018.

(4) Do the same for the third segment: find $V_m(x = 3)$ in terms of $V_0$, the currents in the segments, and the axon resistance.

(5) With $n$ indicating the segment number, does $V_m(x = n)$ increase or decrease as $n$ increases? Does it change more or less rapidly as $n$ increases?


## C. Measurements on your model circuit

**Construct your multi-segment model circuit** (it's convenient to draw the circuit on the whiteboard before constructing it).
> In the previous part, you estimated the ratio of resistance values $R_{mem}/R_{ax}$. Your instructor will assign one group at each lab table to use that estimated ratio and one group to use twice that estimated ratio, so that you can compare circuits built with different ratios.
> Choose resistors with the proper ratio of resistance values, and make sure that you use the same value of resistance for all of the $R_{mem}$ and the same value for all of the $R_{ax}$.
> Use the wires with banana plugs for your connecting wires.
> Make sure that the resistors make good contact with the metal of the stud.


**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. Remember that $V_{mem}(x=0) = V_0$.)
To record voltages in LoggerPro:
> Click on the Data Collection icon, and change the mode to "Events with Entry. "
> Press the start button.
> Use the voltage probe to measure voltages by attaching the probe leads to the circuit just as you would voltmeter leads. 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.
> If a Data Erase box appears, click on Append to Latest Data and proceed.
> Press the stop button.
> To display data points without connecting lines, double click on the graphed data, and unselect the option for connecting the data points.


## Analysis
An equation describing how the voltage across the membrane depends on distance $x$ from the end where a voltage $V_0$ is applied can be found. Mathematically, this is done by following the same line of argument you did in part B, but using infinitesimally short segments of length $dx$, which gives a differential equation for $V_{mem}(x)$. Solving the differential equation gives us the equation we need:

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

$\lambda$ (Greek letter lambda) is called the *length constant* of the axon, and is the distance along the axon at which $V_m(x)$ has decreased by a factor of $e$ (the natural logarithm base e), to 0.37 $V_0$. c is a proportionality constant with units of length that is specific to different types of axons. (This analysis is also known as "cable theory".)

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"

(1) Provide the graph of your data with your best fit function in your lab notebook. Calculate the length constant of your model circuit from your best fit function.

(2) The other group at your table (or at another table) built a circuit with a different value of $R_{mem}$. Compare your graph with theirs and comment on how changing the value of $R_{mem}$ affects the measured distance dependence of $V_m(x)$.

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

(If you don't have time to answer all the questions, you and your lab partner can finish them outside of lab and show them to your lab instructor next week.)

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, as it is for the other resistive circuits that you've studied in this course. 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.

If the ion channels are eliminated from the membrane, what is left is an insulating layer with conducting fluids on each side — a capacitor. The membrane is therefore properly viewed as a resistor and capacitor in parallel, as shown in Fig. 5. As current travels along the axon and enters each new segment, charge also accumulates on the capacitor. It takes 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 $(1-1/e) \approx 0.63$ times its final value as the time constant $\tau = R_{mem}C_{mem}$, where $R_{mem}$ is the resistance and $C_{mem}$ is the capacitance of a segment of membrane. It takes several
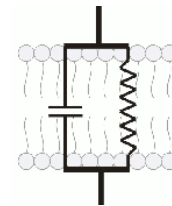


Figure 5. Equivalent circuit for a patch of membrane.

time constants for the membrane potential differences to reach the values that your model resistor circuit gives.

*Speed depends on time constant and length constant: $v \propto \lambda / \tau$*
The speed $v$ at which an action potential travels depends primarily on two things:
> 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 previously. In general, the length constant $\lambda$ is proportional to $\sqrt{\dfrac{R_{mem}}{R_{axon}}}$.

Checking that this result makes sense: If the membrane resistance is very high, or if the axon resistance is very low, current mostly flows *down* the axon, with just a little flowing across the membrane in each successive segment. This leads to a long length constant as given by the equation.
> 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 $\tau = R_{mem}C$.

Faster propagation of nerve impulses confers an advantage to an organism. The next few questions 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 multiplied by a factor of $f$, as shown in Figure 6. The conductivity of the axoplasm does not change.
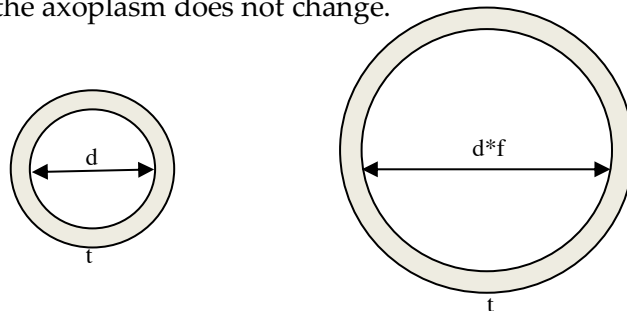


Figure 6. Comparing axons of different diameter.

1. By what factor is $R_{axon}$ multiplied when the diameter is multiplied by $f$?
2. By what factor is $R_{mem}$ multiplied?
3. By what factor is the length constant $\lambda$ multiplied?
4. Show that increasing the axon diameter has *no effect* on the time constant $\tau = R_{mem}C_{mem}$, where $C_{mem}$ is the capacitance of a segment of membrane. *Hint*: You can model the membrane as

a parallel plate capacitor in which the plates have been rolled into a cylinder. Think about why this is a reasonable model.
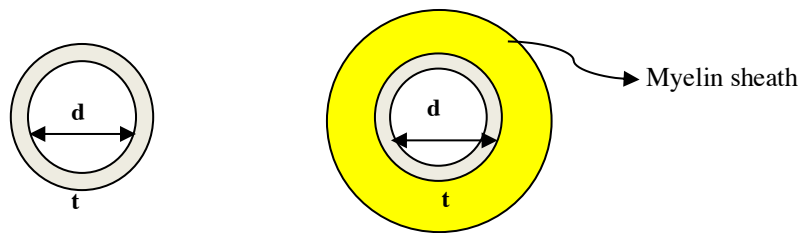
5. By what factor is the signal speed multiplied?

6. To increase the speed by a factor of ten, by what factor *f* would *d* have to change?
This 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 nerve myelination?

Larger diameter 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 10]



**(Fig 10) A myelinated axon**

1. You showed in B2 that increasing the axon diameter has *no effect* on the time constant τ. Argue that assuming that the dielectric constant of the myelin is the same as that of the lipid material of the membrane, the geometry change caused by myelination also has no effect on τ.

2. Let's say that myelination multiplies the membrane resistance by a factor of 1000. By what factor is the length constant multiplied? Why?
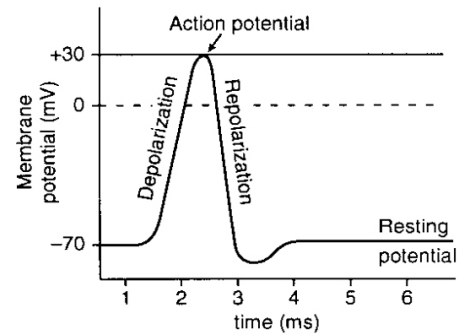
3. Assuming the time constant does not change and the length constant increases as you found in the previous two questions, by what factor is the speed multiplied?

4. Myelination obstructs the membrane's voltage-gated Na⁺ channels that are involved in regenerating the action potential. 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 affects the maximum distance that can exist between these gaps?

5. MS, multiple sclerosis, is a demyelinating disease, which means the axons of neurons are intact, however the myelin sheaths are damaged. Why would loss or damage to the myelin sheath be a problem even if the axon was intact?

## 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.

**(Fig 4) Membrane potential**

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. [Fig 4] 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.