NEUROBIOLOGY 1: EXCITATION

Introduction

This chapter is based on Hodgkin and Huxley's Nobel Prize winning model for the squid axon. Although published over 50 years ago, their model remains today, the paradigm in cellular neurobiology (Hodgkin and Huxley 1952). The model simulates an axon excised from a squid and subjected to experiments such as applying stimuli or clamps, or exposure to drugs or changes in temperature. The model can be used to create experiments to explore a variety of nerve properties, ranging from the classical phenomena of threshold, summation, refractory period, and impulse propagation to more modern concepts of channels, gates, and even molecular events. In addition to faithfully predicting a wide variety of laboratory results, the simulation provides insight about mechanisms of excitation in a way that is not practical with lab experimentation.

Excitation Properties: Threshold, All or None, Refractory Period

If a nerve is stimulated with weak electrical shocks there is a local disturbance but a propagated action potential does not occur. As the stimulus intensity is raised the local disturbance gets larger and finally, at a critical intensity or threshold, an action potential is triggered that propagates along the length of the nerve axon. The amplitude of the action potential is much larger than the local stimulus, and its height does not diminish as it travels along the length of the axon. Further increasing the stimulus strength does not increase the size of the propagated action potential. This is behavior is called all‐or‐none. A recovery phase following excitation, when the axon is not excitable, is called the refractory period.

Building the Model I: The Passive Axon

The electrical excitation characteristics of nerve axons are due to the properties of voltage activated Na^+ and K^+ channels spanning the bilayer membrane. Modeling these characteristics is complex, so we begin with a simpler case: we model the electrical properties of a membrane with channels that are not voltage activated. This can be approximated in the laboratory by applying toxic agents, such as tetrodotoxin (TTX) and tetraethylammonium (TEA), to block voltage activated sodium and potassium channels, respectively.

The excitation behavior of axons arises from the direct effect of the membrane potential on the movements of ions, and from interactions of the membrane potential with the opening and closing of voltage activated membrane channels. We address these complications one step at a time beginning with an axon that is not excitable; thus our simulation corresponds to an axon whose voltage activated Na^+ and K^+ channels have been blocked by TTX and TEA.

The passive axon deals with the first issue: How can movements of diffusing ions within an electric field be described? The electric field (i.e. the force on a unit positive charge) in a membrane is proportional to the more easily measured voltage difference (membrane potential) across the membrane. The field and the membrane potential arise because the membrane is polarized with a net negative charge lining the inner surface and an equal but opposite net positive charge on the

outer surface (see Figure 7 in the Appendix). The relation between membrane potential, *E,* and the charge , *Q*, is simple: as illustrated in Figure 1, E is proportional to Q: $E = (1/C_m)Q$. The reciprocal of the proportionality constant is called the membrane capacitance, C_m.

Equation 1 $$E[volts] = \frac{Q[Coulombs/cm^2]}{C_m[farads/cm^2]}$$

The capacitance, C_m, is a measure of the capacity of the membrane to contain charge. In some ways it is analogous to volume, the capacity of a structure to contain mass. We divide Q by C_m to obtain an intensive 'driving force' E, just as we divide mass by volume to obtain the intensive 'driving force', concentration.

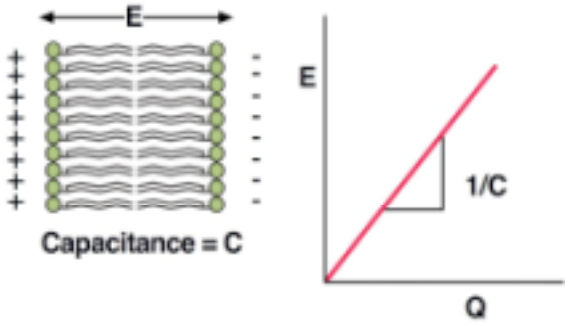


Figure 1. Membrane potentials arise because the membrane is polarized with a net negative charge lining the inner surface and an equal but opposite net positive charge on the outer surface. The relation between membrane potential, E, and charge ,Q, is simple: E is proportional to Q. The reciprocal of proportionality constant is called the membrane capacitance C_m.

Our task is to compute the accumulation of net positive charge Q on the inner surface of the cell membrane after application a stimulating current I_{stim} , or after changes in concentrations or conductances of ions. The membrane potential E is then calculated from Equation 1. For simplicity we assume that the electrical stimulus is delivered uniformly to the entire membrane surface, see Figure 2. This eliminates any spatial coordinate and allows us to focus entirely on time as the independent variable.

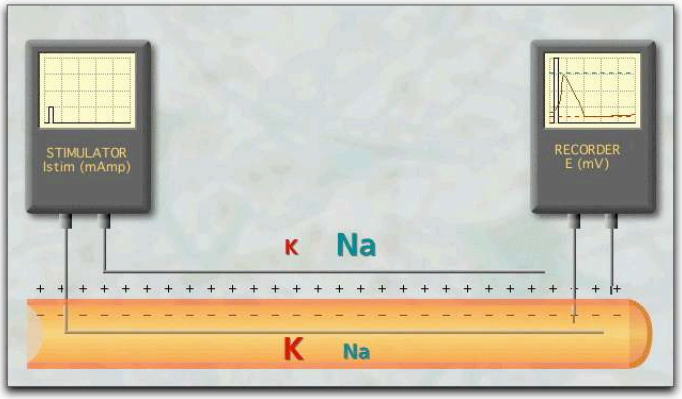


Figure 2. The axon is stimulated uniformly by running the stimulating electrodes along the entire length of the axon, a common experimental design.

The model illustrated in Figure 3 has a reservoir of charge Q on the inner surface (the charge on the outer surface is simply $-Q$). The stimulus I_{stim} represents a current of *positive* charge flowing *into* the cell through the stimulating electrodes, while the membrane current I_{memb} consists of positive charges carried *out* of the cell by Na^+ and K^+. The relatively small amount of additional charge that moves is represented collectively by a leakage current. The membrane potential, $E = Q/C_m$, is the force driving the flow of the three kinds of ions. Computations for the three membrane currents are placed in separate sub programs labeled *Na Current, K Current* and *Leak Current.*

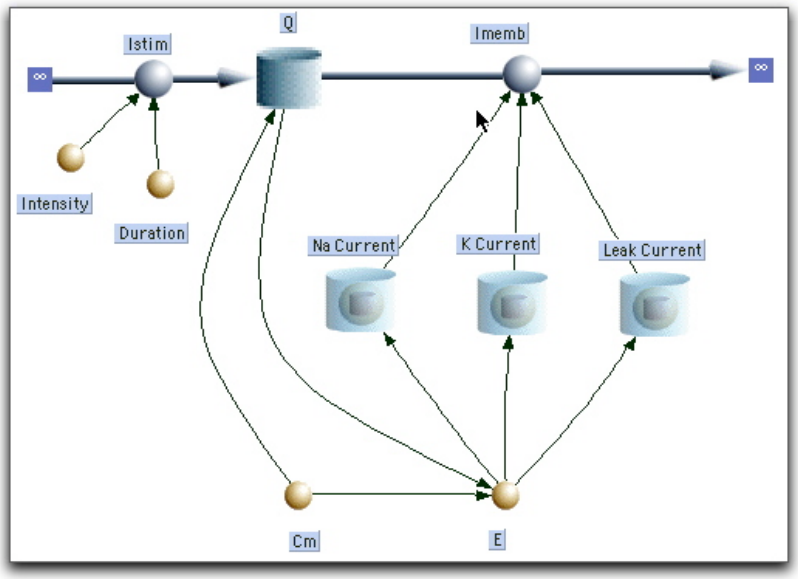


Figure 3 The Hodgkin Huxley excitation model.

Since the concentrations of Na^+, K^+ and other ions are not equal on the two sides of a cell membrane, they tend to diffuse. Since ions carry an electric charge they also tend to drift in response to electric fields generated by charge separations. How can movements of diffusing ions within an electric field be described? We begin by taking up a simple case of equilibrium. Movements will then be described as a response to the departure from equilibrium .

Equilibrium Potentials

Consider the case where a simple, impermeable membrane separates a concentrated and a dilute solution of KCl. Since each solution contains an equal number of positive and negative charges, they are electrically neutral and there is no electrical potential difference between the two (i.e. there is no membrane potential**).** Now patch in some K^+ channels (e.g. gramicidin) that are permeable to K^+ but not permeable to Cl^-.(Figure 4 **).** K^+ begins to diffuse from the concentrated (inside) to the dilute (outside) side.

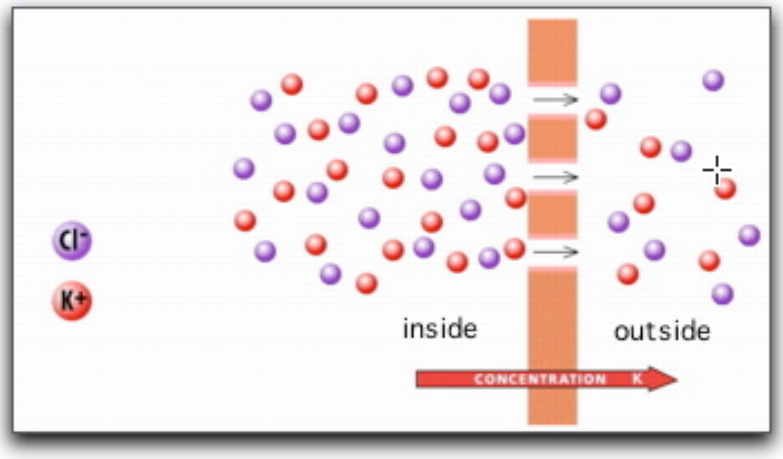


Figure 4 K+ channels are patched into a lipid bilayer membrane that separates two KCl solutions. The solution on the left (inside) is more concentrated.

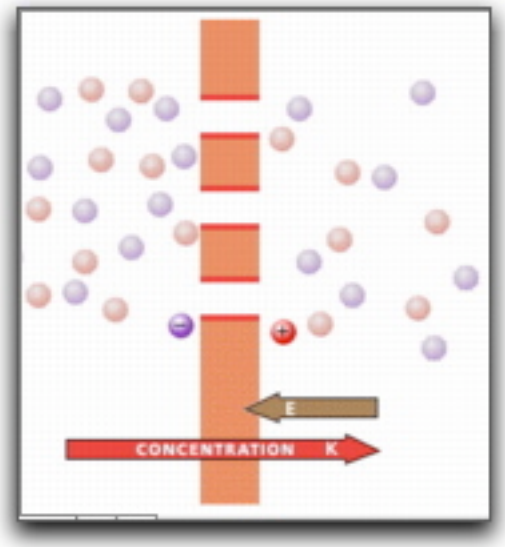


Figure 5 K^+ diffusion separates charge.

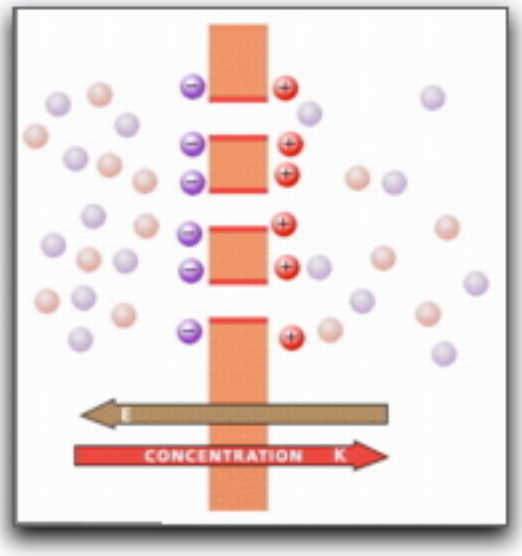


Figure 6 Equilibrium: Diffusion drift is balanced by electrical retardation

As each K^+ ion moves across the membrane it delivers a positive charge to the dilute outside and leaves an uncompensated negative Cl□ ion behind on the inside. This makes it more difficult for the next K^+ to move across to the right because it is repelled by the excess + ions on the right and attracted to the excess □ ions on the left. In other words an electrical force opposing the diffusion begins to develop. Thus a membrane potential arises with the concentrated solution negative (Figure 5).

The more ions that diffuse separating more positive and negative charges, the more polarized the membrane becomes and the larger the retarding electrical force. Finally the electric force (E arrow) is just equal and opposite to the concentration gradient (K^+ arrow). At this point the tendency for

K^+ ions to diffuse from left to right is balanced by the tendency of K ions to drift from right to left due to electrical forces. The K^+ ion is in equilibrium. The magnitude of the membrane potential at this point is called the equilibrium potential for K^+, or E_{K^+} . This equilibrium state is attained very rapidly because very few ions have to move to produce large electrical forces. The polarizing ions (i.e. those that are in excess on either side of the membrane) are confined to a narrow layer adjacent to the membrane. (The width of this layer is less than 10 nm.) Beyond this thin layer, the bulk solutions on either side of the membrane are electrically neutral

The larger the concentration difference between the two solutions, the larger the equilibrium potential. In a way it is an electrical measure of the concentration 'force', and it is important for our purposes because it allows a meaningful comparison of the actual electrical force with the concentration 'force'. The equilibrium potential E_K for potassium is determined by the ionic concentrations inside and outside of a cell as (Hille 1992; Benedek and Villars 2000):

Equation 2 $$E_K = E_{in} - E_{out} = -\frac{RT}{F}\ln\frac{K_{in}}{K_{out}}$$

where E_{in}, E_{out}, K_{in}, K_{out} are the electrical potential and K concentration on the inside and outside, while *R, T,* and *F* are the gas constant, absolute temperature, and Faraday constant respectively.

Each ion species will be present at different concentrations and accordingly will require a different value for E if they are if they are to be held in equilibrium. The equilibrium potential for Na^+ is

Equation 3 $$E_{Na} = E_{in} - E_{out} = -\frac{RT}{F}\ln\frac{Na_{in}}{Na_{out}}$$

Ionic Currents Are Proportional to Their Departure from Equilibrium

Using nominal concentrations inside and outside squid axons together with Equation 2 and Equation 3, we arrive at $E_K = -77$ and $E_{Na} = 50$ mv. Neither ion is normally in equilibrium, so the actual membrane potential, *E*, is neither of these. The difference $E - E_K$ is a measure of the departure of K from its equilibrium state and we assume the it's flow is proportional to this departure i.e.

Equation 4 $$I_K = g_K(E - E_K)$$

where I_K denotes the potassium current and g_K is a proportionality constant called the potassium conductance. Flow of K^+, J_K, can be measured in terms of moles $\cdot sec^{-1} \cdot cm^{-2}$, but here we describe it by the flow of positive charge that it carries, i.e. the potassium current, I_K,. The two flows are related by the Faraday constant (96,487 Coulombs$\cdot mol^{-1}$, see Table 2) i.e.

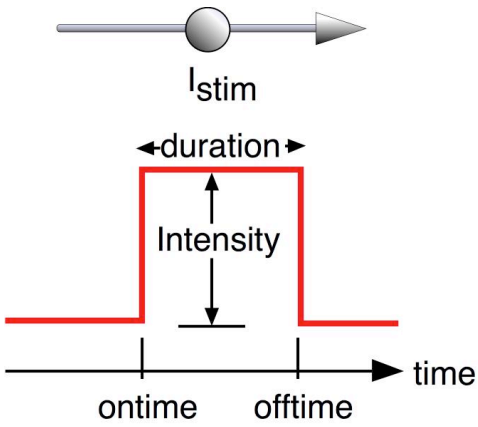
Equation 5 $$I_K[amp \bullet cm^{-2}] = F[Coulombs \bullet mol^{-1}] \bullet J_K[mol \bullet \sec^{-1} \bullet cm^{-2}]$$

Applying the same arguments to the other ions, we have

Equation 6 $$I_{Na} = g_{Na}(E - E_{Na})$$

Equation 7 $$I_L = g_L(E - E_L)$$

where g_{Na} and g_L are the sodium and 'leak' ion conductances. By convention, *positive current is always defined as positive charge moving out of the cell*. The conductances (= 1/resistances are proportional to the number of channels available to that ion species. The equilibrium potential of any ion is the value of the membrane potential (inside − outside) that would be required to prevent any diffusion of that ion across the membrane. They are a measure of the concentration ratio on the two sides of the membrane.



In addition to the ion currents the experimenter may impose a stimulating flow of positive charge *into* the cell. If this imposed current has the form of a square wave, it has the form:

Equation 8 $$I_{step} = Intensity*SQUAREPULSE(on\ time,\ duration)$$

The Berkeley Madonna function *SQUAREPULSE* is either 0 or 1, so that the *Intensity* specifies the height of the pulse. The off time is not specified, instead the *duration* of the pulse is required.

Building the Passive Axon

In the passive axon model, the conductance of each ion remains constant; i.e. it does not depend on voltage and it does not change with time. Using the data in Table 1 together with the model of Figure 3:

1. Simulate the response of the membrane potential *E* when you stimulate the cell with a square wave of 100 µamps with a 10 msec. duration. An easy way to set up the submodels is to create a formula icon (round ball) for each of the three currents I_K, I_{Na}, and I_L. Connect an arrow from *E* to each of these icons and other arrows from the icons to I_{memb}. Now, for example, select I_K and then select the menu item *Flowchart >Group,* and label the submodel icon *K current* Open the submodel window, create formula icons for E_K and g_K, and complete the submodel flowchart for I_K. Do the same for the other currents. And insert E_r / C_m for the initial value of Q. When the model is compete, run it and plot *E* and I_{stim} vs. time**.** Note that the system is linear: it depends only on the first power of *E*. The cell responds with a single time constant. What is its magnitude? Is the response all or none? Is there a threshold?

2. Illustrate the sensitivity of the system (speed of response and final steady state resting potential) when you change the parameters listed below. An easy way to do this is to use the *Batch Runs* command. Begin by making simultaneous plots of 0.1, 1. 5, and 25 times the normal value given in the table for each parameter. This can be done by choosing *Geometric Series* and setting the maximum and minimum values for the specific parameter you are investigating. As you see your results you may want to change these values to illustrate some particular point. Parameters to change are the membrane capacity, C_m, the number of open sodium and potassium channels, g_{Na} and g_K.

3. Set $I_{stim} = 0$ and assume that by some means the resting potential has been set to zero ($E = 0$ at *time* = 0); i.e. the membrane has been 'short circuited'. Suddenly the short is removed and the membrane is allowed to charge up to its normal resting potential.

3-1 Simulate the time course of this experiment.

3-2 Compare the amounts of charge carried by Na^+, K^+, and L^+ required to charge up the membrane

3-3 How much charge in Coulombs moved in to accomplish this? Using the Faraday constant, translate this into moles of positive ions.

3-4 Show that no matter where you place the initial value of the membrane charge (and corresponding membrane potential) the potential always returns to the same value.

3-5 Show that changing the capacitance C_m will change the speed of response but will not effect the final value. How does the speed of response (increase/decrease) when C_m increases?

3-6 Show that changing the conductances *in the same proportion* (i.e. increase all of them by a factor of 10x) will also change the speed of response but will not effect the final value. How does the speed of response (increase/decrease) when conductances increases?

QUANTITY	SYMBOL	UNITS	VALUE
membrane capacity	C_m	μfarad/cm^2	1
Equilibrium potential for K^+	E_K	mV	−77
Equilibrium potential for Na^+	E_{Na}	mV	50
Equilibrium potential for L^+	E_L	mV	−54.4
resting potential	E_r	mV	−65
K^+ conductance	g_K	mmho/cm^2	0.425
Na^+ conductance	g_{Na}	mmho/cm^2	0.0167
leakage conductance	g_L	mmho/cm^2	0.3 mmho/cm^2

Table 1. **Data for the passive axon.**

Save the model! You will use it next time to patch in voltage activated channels and simulate nerve excitation.

Appendices

Electrophysiological definitions

DEFINITION	Symbol	Value
Avogadro's Number	N	6.02×10^{23} mol^{-1}
Faraday's constant	F	9.65×10^{4} C/mol
elementary charge	e	1.602×10^{-19} C
gas constant	R	8.315 J/(mol·°K)
joule	J	1 V/C
volt	V	1 J/C
ampere	A	1 C/s

Table 2. Some electrophysiological constants.

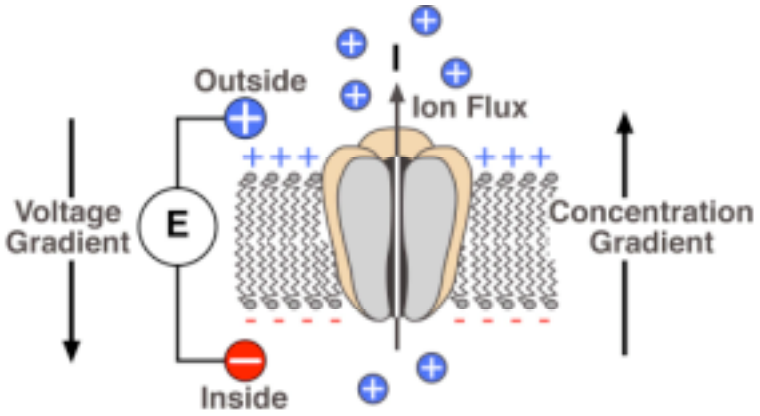


Figure 7. Transferring 1 mole of univalent ions (e.g. K^+) across a membrane in 1 second amounts to a current equal to Faraday's constant. 1 Joule of energy is required to separate 1 Coulomb of charge across a potential of 1 volt.

References

Benedek, G. and F. Villars (2000). *Physics. With Ilustrative Examples From Medicine and Biology*. New York, Springer-Verlag.

Hille, B. (1992). *Ionic Channels of Excitable Membranes*. Sunderland, MA, Sinauer.

Hodgkin, A. and A. Huxley (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol (Lond.) **117**: 500-544.