Neurobiology 1

Introduction

This chapter begins with 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, exposure to drugs or changes in temperature. The model can be used to design 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 model provides insight into mechanisms of excitation in a way that is not practical with lab experimentation. The latter portion of the chapter is devoted to updating the model, bringing it more in line with modern views of ion channel structure and function.

Axon excitation is characterized by threshold, all-or-none, and 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 increases until, 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 amplitude 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 follows excitation during which the axon is not excitable; this is called the **refractory** period.

Building the HH model I: The passive axon

The behavior of nerve axons arise from the properties of voltage activated Na^+ and K^+ channels that span 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 the toxic agents, tetrodotoxin (TTX) and tetraethylammonium (TEA), to block voltage activated sodium and potassium channels, respectively.

Membrane capacitance is a measure of the 'capacity of the membrane to contain charge

The passive axon model deals with a basic question: How to describe the movements of diffusing ions within an electric field? The electric field can be defined as the force on a unit positive charge. In a membrane, the field is proportional to the more easily measured voltage difference, or **membrane potential**, across the membrane. The electric field and the membrane potential arise because the membrane is polarized: a net negative charge lines the inner surface and an equal

opposite net positive charge lines the outer surface. The relation between the membrane potential, V_m, and the net charge, Q, is simple: as illustrated in **Figure 1**, V_m is proportional to Q. The reciprocal of the proportionality constant is called the **membrane capacitance**, C_m.

Equation 1 $$V_m[volts] = \frac{Q[Coulombs/cm^2]}{C_m[farads/cm^2]}, \text{ or } Q = C_m \cdot V_m$$

C_m measures the capacity of the membrane to hold charge on its surfaces. Thus it is analogous to 'volume', which is the capacity of a container to hold mass. If we divide Q by C_m, we obtain an intensive 'driving force' V_m, just as we divide mass by volume to obtain an intensive 'driving force', concentration.

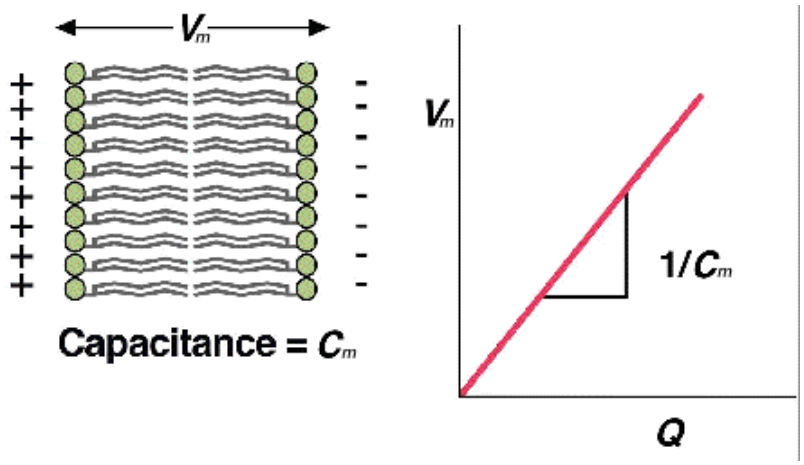


Figure 1. The charge a membrane accumulates on its surfaces is proportional to the voltage imposed across the membrane: $Q = C_m \cdot \Delta V_m$. The proportionality constant is called the membrane capacitance.

The electrical stimulus is delivered uniformly to the entire membrane surface

Our task is to compute the accumulation of net positive charge, Q, on the inner surface of the cell membrane after applying a stimulating current, I_{stim}, or after changing the concentrations or conductances of ions. The membrane potential, V_m, is then calculated from Equation 1.

For simplicity we assume that the electrical stimulus is delivered uniformly to the entire membrane surface. This can be accomplished by using a long axial electrode as shown in Figure 2. Uniform stimulation eliminates any spatial dependence and allows us to focus entirely on *time* as the independent variable.

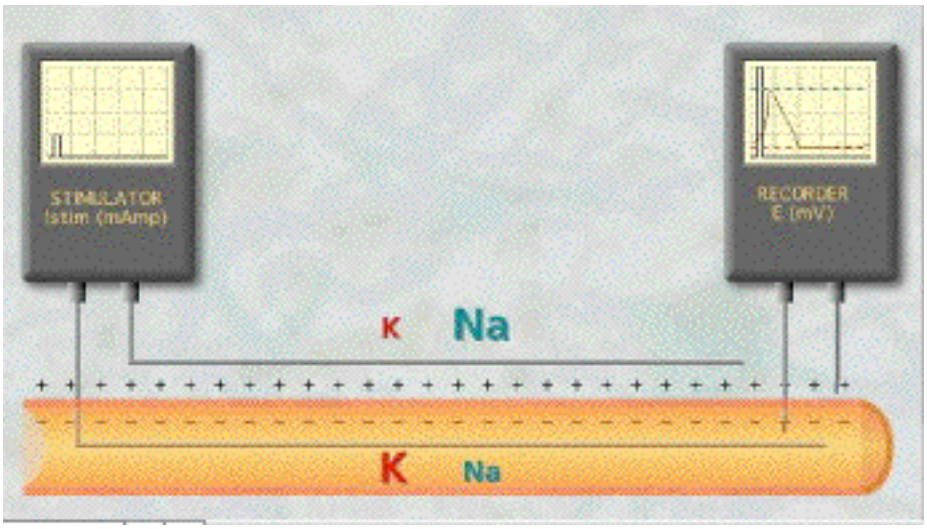


Figure 2. The axon is stimulated uniformly by running the stimulating electrodes along the length of the axon, a common experimental design.

A generic model for passive current flow across cell membranes

The model illustrated in Figure 3 shows a reservoir of charge Q on its 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_m, consists of positive charges carried out of the cell by Na^+ and K^+. A small amount of additional charge moves with these ions, and is collectively represented by a leakage current. The membrane potential, $V_m = Q/C_m$, is the driving force for the flow of the three classes of ions. Computations for the three membrane currents are placed in separate windows (sub programs) labeled *Na Current, K Current* and *Leak Current.* Let V_r denote the resting membrane potential; we compute the initial value for Q by inserting into the dialog of the Q icon

Equation 2 $$Q = \frac{V_r}{C_m} \qquad at\ time = 0$$

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 the ions carry an electric charge they also drift in response to electric fields generated by any charge separation. How can movements of diffusing ions within an electric field be described? We begin by taking up the simplest case: equilibrium. Subsequently, ion currents will be described as a response to the departure from equilibrium.

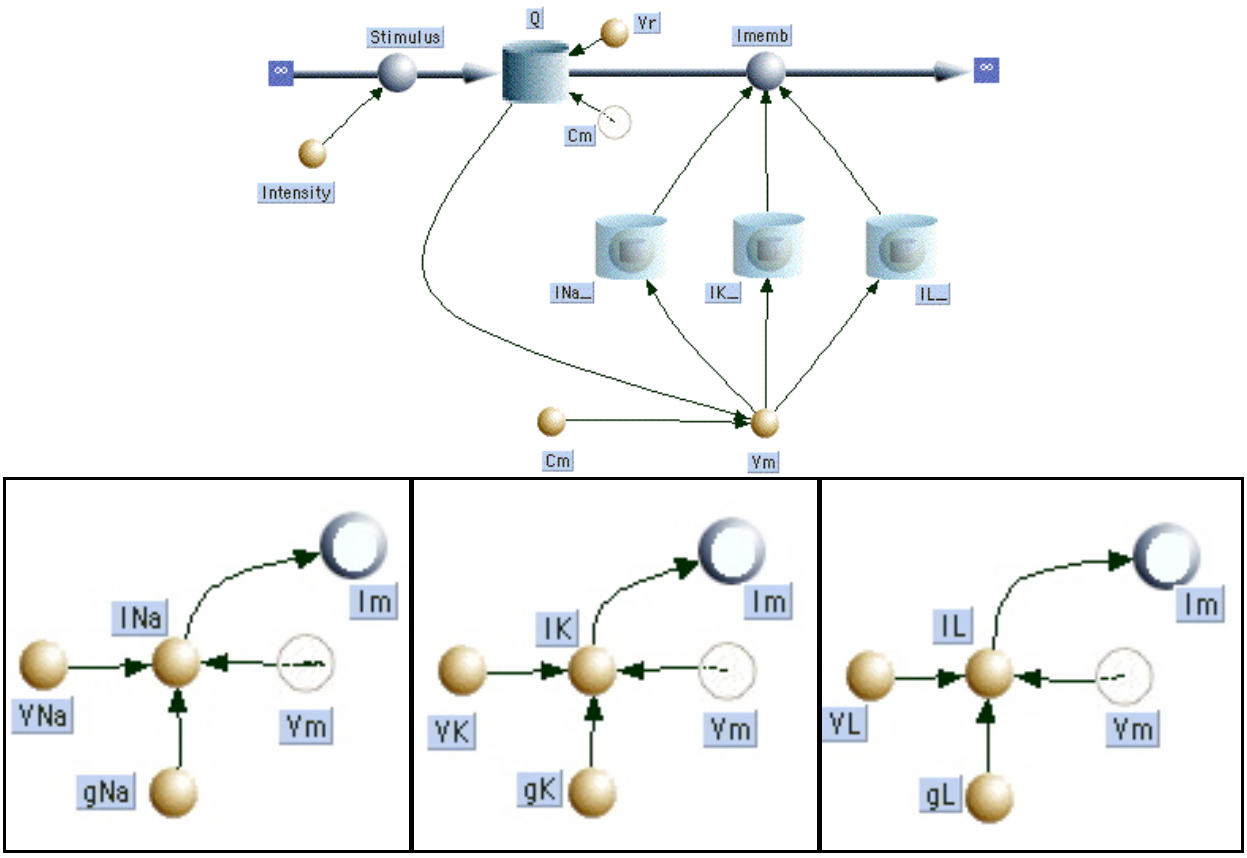


Figure 3 (a) Model for the flow of Na^+ and K^+ currents across a cell membrane. Submodels for Na^+ (b), K^+ (c), and leakage (d) currents.

Equilibrium membrane potentials reflect ion concentration gradients

Consider the case where a membrane, impermeable to ions, 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, so there is no electrical potential difference between the two (V_m = 0**).** Now patch in some channels that are permeable to K^+ but not permeable to Cl^- (e.g. gramicidin) , shown in Figure 4a**.** K^+ will begin to diffuse from the concentrated (inside) to the dilute (outside) side.

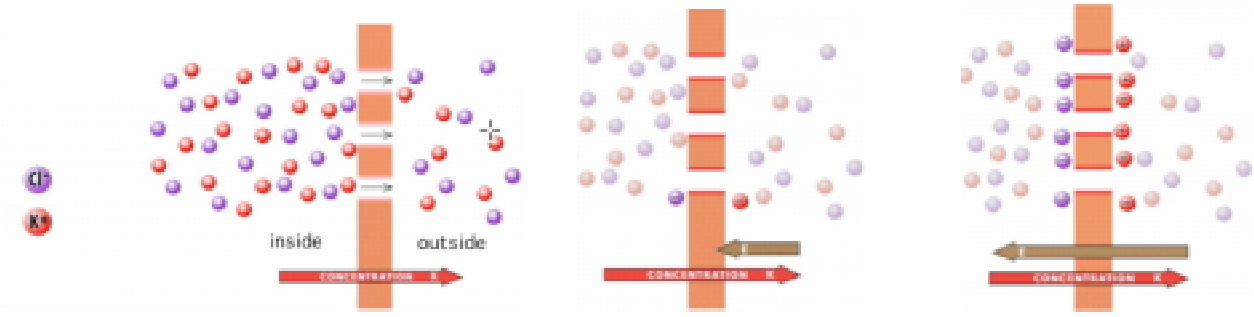


Figure 4 (a) K^+ channels are patched into a lipid bilayer membrane that separates two KCl solutions. The solution on the left (inside) is more concentrated. (b) K^+ diffusion separates charge. (c) Equilibrium: Diffusion drift is balanced by electrical retardation

As each K^+ ion moves across the membrane it delivers a positive charge to the dilute solution 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 to the right and attracted to the excess (–) ions on the left (Figure 4b). In other words, an electrical force opposing the diffusion begins to build up, that is reflected in a membrane potential with the concentrated solution negative .

The more ions that diffuse through the channels, the more polarized the membrane becomes (because of the separation of positive and negative charges), and the larger becomes the retarding electrical force. Finally, the electric force (E arrow in Figure 4c) 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, and the K^+ ion is in equilibrium. The magnitude of the equilibrium membrane potential is called the **equilibrium potential** for K^+, or V_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 less than 10 nm thick adjacent to the membrane. 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, V_K, for potassium is determined by the ionic concentrations inside and outside of a cell as (see the Appendix)

Equation 3 $$V_K = V_{in} - V_{out} = -\frac{RT}{F}\ln\frac{K_{in}}{K_{out}}$$

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

Each ion species will be present at different concentrations and so will require a different value for V_m if they are if they are to be held in equilibrium. The equilibrium potential for Na^+ has the same form, but with Na^+ replacing K^+:

Equation 4 $$V_{Na} = V_{in} - V_{out} = -\frac{RT}{F}\ln\frac{N_{in}^+}{Na_{out}^+}$$

Ionic currents are proportional to their departure from equilibrium

Using typical concentrations inside and outside squid axons in Equation 3 and Equation 4, we arrive at $V_K = -77$ and $V_{Na} = 50$ mV. Neither ion is normally in equilibrium, so the actual membrane potential, V_m, is neither of these. The difference $V_m - V_K$ is a measure of the departure of K^+ from its equilibrium state and we assume that its flow is proportional to this departure, i.e.

Equation 5 $$I_K = g_K(V_m - V_K)$$

where I_K denotes the potassium current and g_K is a proportionality constant called the **potassium conductance**. The flow of potassium ions, denoted by J_K, can be measured in moles/sec/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·mol^{-1}) i.e.

Equation 6 $$I_K[amp \cdot cm^{-2}] = F[Coulombs \cdot mol^{-1}] \cdot J_K[mol \cdot \sec^{-1} \cdot cm^{-2}]$$

Applying the same arguments to the other ions, we have

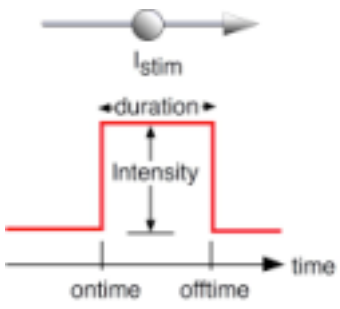
Equation 7 $$I_{Na} = g_{Na}(V_m - V_{Na})$$

Equation 8 $$I_L = g_L(V_m - V_L)$$

where g_{Na} and g_L are the sodium and 'leak' ion conductances. The conductances (= 1/resistance) are proportional to the number of channels available to that ion species. Equation 5, Equation 7, and Equation 8 are written in accordance with the convention that *positive current is defined as positive charge moving out of the cell*.

Electrical stimuli deliver positive charge to the inner membrane surface

In addition to ion currents, an 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 9 $I_{stim} = Intensity*SQUAREPULSE(on\ time, duration)$

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.

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Q[nCoul], V_m[mV], C_m[μf], I[μamps], g[mmho] and time[msec] form a consistent set of practical units

Consider a patch of membrane with area = 1cm ^{2}. From Equation 1, Q [Coul] = C [farads]·V [volts]. But, farads and volts are much too large to be convenient in physiology, so we use microfarads [μf] and millivolts [mV] instead. However, if we measure C_m in μf and V_m in mV, then we must use Q in nano-Coulombs (1 nCoul = 10^{-9} Coul) because, after substituting 1V = 10^3 mV and 1 farad = 10^6 μfarads into the above, we find

$$\text{Coul} = 10^6\ \mu\text{farads}\cdot 10^3\ \text{mV} = 10^9\ \mu\text{farads}\cdot\text{mV}$$

$$10^{-9}\ \text{Coul} = \text{nCoul} = \mu\text{farads}\cdot\text{mV}$$

Thus a consistent set of units is:

$$Q\ [\text{nCoul}] = C\ [\mu\text{f}]\cdot V\ [\text{mV}]$$

If we measure time in msec, then current will be in

$$\text{nCoul}\cdot\text{msec}^{-1} = \mu\text{Coul}\cdot\text{sec}^{-1} = \mu\text{amp}$$

Even though the 'real' units, nCoul·msec^{-1}, define the time scale for the model, it is conventional to refer to current units by its more conventional equivalent, μamps.

Conductance is defined by Ohms law:

$$I\ [\text{Coul}\cdot\text{sec}^{-1}] = g\ [\text{mho}]\cdot V_m\ [\text{volt}],$$

where the conductance, g [mho] = 1/resistance [ohm]. If we use the units current (μamps) and time (msec), then

$$\text{amps} = 10^6\ \mu\ \text{amps} = \text{mho}\cdot\text{volts} = \text{mho}\cdot 10^3\ \text{mV}$$

$$\mu\ \text{amps} = 10^{-6}\ (\text{mho}\cdot 10^3\ \text{mV}) = 10^{-3}\ \text{mho}\cdot\text{mV} = \text{mmho}\cdot\text{mV}$$

So, the units of conductance are *mmho* .

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Building the passive axon model

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 V_m 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 V_m 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 *IK_*. Open the submodel window, create formula icons for V_K and g_K, and complete the submodel flowchart for I_K. Do the same for the other currents, and insert V_r / C_m for the initial value of Q. When the model is compete, run it and plot V_m and I_{stim} vs. time**.** Note that the system is linear: it's flow depends only on the first power of V_m. 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, and 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 (V_m = 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 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.

3-3 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?

3-4 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?

3-5 Compare the amounts of charge carried by Na^+, K^+, and L^+ required to charge up the membrane. You can accomplish this by placing a connecting a new flow to a new reservoir for each ion in its current submodel window.

3-6 How much charge in Coulombs moved in to accomplish this? Using the Faraday constant, translate this into moles of positive ions.

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

QUANTITY	SYMBOL	UNITS	VALUE
membrane capacity	C_m	μfarad/cm^2	1
Equilibrium potential for K^+	V_K	mV	−77
Equilibrium potential for Na^+	V_{Na}	mV	50
Equilibrium potential for L^+	V_L	mV	−54.4
resting potential	V_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

Building the HH Model II: Voltage Activated Channels

Models for voltage activated channels generally assume that proteins forming the channel undergo conformational changes when subjected to strong electrical fields. Some conformations create open channels permitting specific ions to pass through the membrane while others are closed. A common metaphor visualizes channels as canals containing charged gates that open or close when electric fields change (see Figure 5). The reality is much more complicated, but this simple picture will suffice for our model. Hodgkin and Huxley had little idea of what the ion channels looked like; today we know much more (Berg, Tymoczko and Stryer 2002), but their model is still an accurate description of the dynamics.

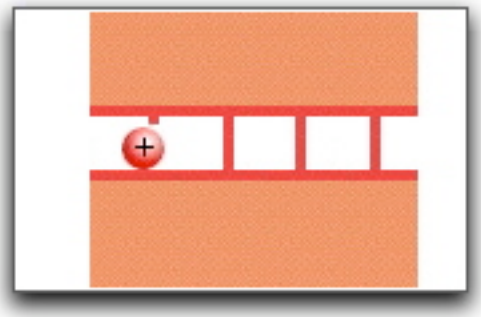

Figure 5 Closed channel with 4 gates, one open, three closed. The channel is open only when all four gates are open.

Single gate channels are not sufficient

We begin our analysis with the simplest case, a population of K^+ channels that contain a single gate. Our goal is to compute the fraction, *n*, of these channels that are open at any time. The first stage of the model is shown in Figure 6, where the reservoir is the variable *n*. The rate of open

channel formation, R_{open}, is proportional to the fraction of closed channels (an open channel can only be formed from a closed channel), but the fraction of closed channels is equal to $1 - n$. Therefore,

Equation 10 $$R_{open} = a_n \cdot (1 - n)$$

where a_n is a rate constant. Similarly, the rate of closed channel formation, R_{close}, is proportional to the fraction, n, of open channels. With b_n denoting the rate constant for closing, we have

Equation 11 $$R_{close} = b_n \cdot n$$

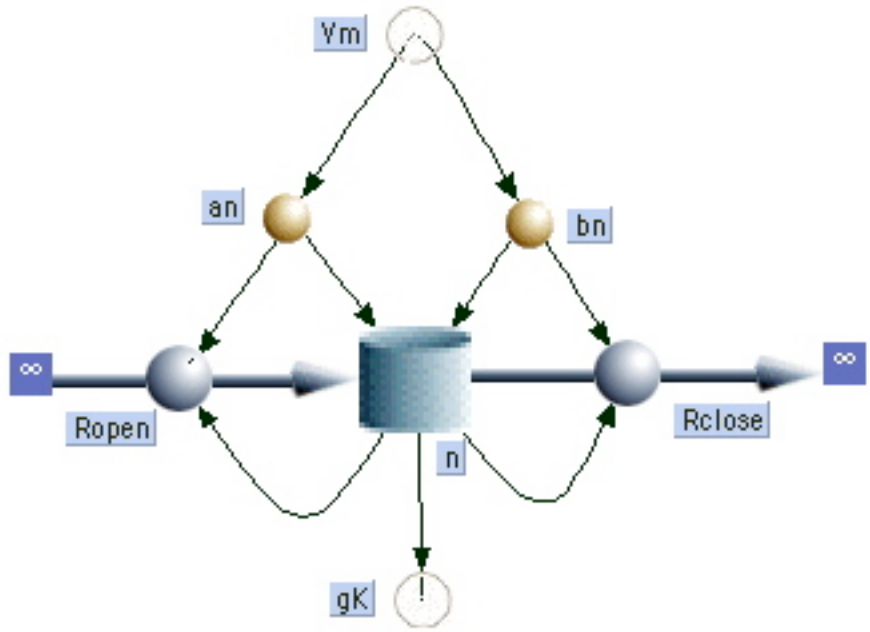

Figure 6 Model for a channel gate: $n_{open} \underset{b}{\overset{a}{\rightleftharpoons}} n_{closed}$

The initial condition for n at rest (n not changing and $V_m = V_r$) is given by the steady state condition $R_{open}=R_{closed}$:

Equation 12 $$n_{initial} = \frac{a_n}{a_n + b_n} \qquad at\ V_m = V_r$$

The dependence of the initial condition on the rate constants requires the additional arcs from a_n and b_n to n shown in Figure 6. Note that n ranges from 0 to 1 and can be viewed as the *probability* of finding an open gate.

Thermal fluctuations cause individual gates to open and close randomly. The rates of these open↔close transitions will depend on the electrical field that changes the energy level of the gate and/or lowers the activation energy required for a transition. This dependence is indicated in

Figure 6 by the arcs linking a_n and b_n to the membrane potential V_m (see Equation 15 for the explicit dependence). Finally, n is connected to g_K using the relation: $g_K = g_{K\max} \cdot n$, where g_{Kmax} is the conductance when all channels are open, i.e. if each channel has conductance g_{Kss} and there are a total of N_T channels (open + closed), then $g_{Kmax} = g_{Ks} \cdot N_T$. The single gate model does not account for the experimental data on squid axon. However, the analysis, given above, paves the way for introducing multiple gates that do account for the data

Multiple gate channels are required

The simplest multiple gate channel model assumes that all gates act independently of each other, In this case, our computation of n, the fraction of open gates, is still valid. If n is the probability of finding a channel with one gate open, then the probability of finding a channel with two open gates is given by n^2, and the probability of finding r gates open is n^r. In general we can write

Equation 13 $$g_K = g_{K\max} \cdot n^r$$

Figure 7 shows a sample of the data used to test various gate models. The results show K^+ conductance measurements on a squid axon that was subjected to a sustained depolarization: the membrane potential was raised from its resting value of –65 mV to –2mV and held constant at –2 mV for about 11 msec. Clearly, using a single gate per channel (r = 1) is inadequate, but setting r = 4 fits the data nicely. We conclude that there are 4 gates in the K^+ channel and use

Equation 14 $$g_K = g_{K\max} \cdot n^4$$

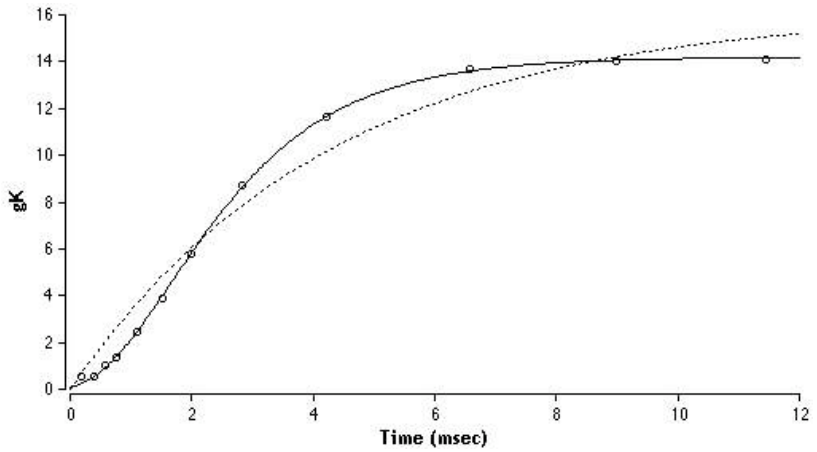

Figure 7. Potassium conductance of a squid axon subjected to a voltage clamp where, at time = 0, the membrane potential was raised from its resting value of –65 mV to –2mV and held constant at that level for 12 msec (Hodgkin and Huxley 1952). Experimental data (circles) are compared to 'best fit' model predictions (Equation 13) with r = 1 (dashed curve) and r = 4 (solid curve).

In general, the values of a_n and b_n in Equation 12 will depend on the membrane potential, V_m. The following empirical equations give values for a_n and b_n:

Equation 15

$$a_n = \frac{0.01 \bullet (V_m + 55)}{1 - \exp\left(\frac{-(V_m + 55)}{10}\right)} \qquad b_n = 0.125 \bullet \exp\left(\frac{-(V_m + 65)}{80}\right)$$

Equation 15 introduces voltage activation into the model. The correct values to use in the above equation for the initial value of *n* correspond to $V_m = V_r$. (This will automatically occur if you don't start the stimulus at $t = 0$; i.e. make sure that *ontime* > *0*.).

The model for an axon with voltage activated K^+ channels can be constructed using Figure 6 together with Equation 10, Equation 11, Equation 12, Equation 14, and Equation 15 within the K^+ *current* submodel window of Figure 3. (Remember: double click on the submodel icon to open its window).

Exercises

1. Begin by running your simulation for 8 msec with a time step DT = 0.03 msec. Use a SQUAREPULSE stimulus with *intensity* =100, *ontime* =1, and *duration* = 0.3. Plot the stimulus and membrane potential on the same graph.

2. Increase the size of the stimulus. Is there a threshold? Is the response 'all or none'?

3. Increase the *intensity* to 500, and include the number of open K channels (either n^4 or g_K) in your plot. You can see from the plot how n^4 or g_K lags behind the stimulus; the K gates are slow to respond. Following the stimulus, notice how V_m dips below its resting value only to return to it several msec later. This same dip is seen in fully formed action potentials and is due to the slow response of the K gates: they are not only slow in responding to a depolarization, they are also slow in returning to their normal configuration once the depolarization is removed.

4. Set *intensity* to 500 and the *duration* to 5 msec. V_m rises and then falls as the K^+ gates open allowing more K^+ to move down its concentration gradient , out of the axon. Evidence of open K^+ gates is also seen when the stimulus is turned off. Now the descent of V_m overshoots the resting potential and slowly returns to normal. As we shall see, the persistence of open K^+ channels immediately following the stimulus is a major contributor to the refractory period.

5. To make it easier to take out, or patch in, the voltage activated K^+ channels, modify the expression for g_K in the following way. Connect a new formula icon called *SwitchK* to the *gK* icon. Remembering that for the passive axon $g_K = 0.425$ mmho/cm^2 (Table 1 of that exercise), we can redefine g_K as

Equation 16

$$gK = IF\ SwitchK = 0\ THEN\ 0.425\ ELSE\ gK \max * n^4$$

Now, by simply setting each *SwitchK* = 1 we simulate an axon with voltage activated K^+ channels present. With *SwitchK* = 0, we simulate an axon with passive (non voltage activated) K^+ channels.

Na+ channels have fast and slow gates

Now we add voltage activated Na^+ channels. The treatment is very similar to the K^+ channels. As before, each channel contains four gates, but only three of them (the fast m-gates) are identical ; they tend to open when the membrane is depolarized. The fourth (slow h gate) *closes* upon depolarization. All four gates have to be open simultaneously before Na^+ can pass through one of these channels. Na conductance is described by[1]

Equation 17 $$g_{Na} = g_{Na\,\max} \cdot m^3 \cdot h$$

- $g_{Na\ max}$ = maximal Na^+ conductance = 120 mmho/cm^2 (constant)
- m = the fraction of the total number of *fast* Na^+ gates that are open at any time.
- h = the fraction of the total number of *slow* Na^+ gates that are open at any time.
- m^3 and h vary between 0 and 1 and depend on both V_m and *time*.

They accumulate as follows:

	$\mathbf{R_{open}}$	$\mathbf{R_{close}}$
m	$a_m(1-m)$	$b_m m$
h	$a_h(1 - h)$	$b_h h$

Table 2. Rates for m and h. The a's and b's are rate constants defined in Equation 19 and Equation 20. The m and h gates submodels are similar to the n-gates model.

As in the case of the K^+ channel, we have to supply initial values for m and h. These are obtained by assuming that, prior to stimulating, the nerve has been in a steady (resting) state with $V_m = V_r$. Therefore, $R_{open} = R_{close}$ for both m and h. Using Table 3 above to apply these criteria, we can solve for initial values as

Equation 18 $$m_{initial} = \frac{a_m}{a_m + b_m}, \qquad h_{initial} = \frac{a_h}{a_h + b_h}, \qquad at\ V = V_r$$

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[1] The notation m, h, and n for the Na^+ and K^+ channels is admittedly not very mnemonic. However, time has sanctified the notation, and that is how they are denoted in virtually all textbooks. Unfortunately, we are bound to follow precedent here.

In general, the values of the *a's* and *b's* depend on V_m. Again, the correct values to use in the above equations correspond to $V_m = V_r$. and this will automatically occur if you don't start the stimulus at $t = 0$; i.e. make sure that *ontime* > *0*.

The *a's* and *b's* change with V_m as it departs from V_r. They are obtained from the following empirical expressions :

Equation 19

$$a_m = \frac{0.1 \bullet (V_m + 40)}{1 - \exp\left(\frac{-(V_m + 40)}{10}\right)} \qquad b_m = 4.0 \bullet \exp\left(\frac{-(V_m + 65)}{18}\right)$$

Equation 20

$$a_h = 0.07 \bullet \exp\left(\frac{-(V_m + 65)}{20}\right) \qquad b_h = \frac{1.0}{\exp\left(\frac{-(V_m + 35)}{10}\right) + 1}$$

Just as in the case of the K^+ channels, you can modify the expression for g_{Na} in your simulation to make it simple to take out the voltage activated Na^+ channels or patch them back in at your convenience. Introduce a new formula icon called *SwitchNa*, and connect it to g_{Na} . For the passive axon g_{Na} = 0.0167 mmho/cm^2, so we redefine g_{Na}as

$$g_{Na} = IF\ SwitchNa = 0\ THEN\ 0.0167\ ELSE\ g_{Na\max} \cdot m^3 h$$

With *SwitchNa* = 1, voltage activated Na channels are active; with *SwitchNa* = 0, they are absent.

Stimulate the axon

Leaving both Na and K voltage activated channels in your model should be sufficient to reproduce most of the excitation behavior of real axons.

1. As before, run your simulation for 8 msec with a *dt* = 0.03 msec. Use a *squarepulse* stimulus with *intensity* =100, *ontime* =1, and *duration* = 0.3. At first plot the stimulus and membrane potential on the same graph.

2. Once you have succeeded in obtaining an action potential experiment with:

a. the *intensity* (e.g. reverse it)

b. the *duration*

c. the shape of the stimulus (e.g. a ramp or an alternating current sine wave)

d. two stimuli in a row

3. See if you can reproduce some of the classical results described in text books. E.g.

a. threshold

b. all or none behavior

c. refractory period

d. accommodation

4. You are now in a position to explore the details of nerve excitation in depth by plotting any of the variables you choose. The Na^+ and K^+ currents yield the actual flow of these ions at each instant. The conductances are a measure of the number of open channels at each moment. m^3 is a measure of the fraction of fast Na^+ gates that are open, while h measures the fraction of slow gates. You can measure the actual amount of Na^+ that moves in with each impulse and estimate how fast the Na^+ pump would have to operate to pump it back out during rest. You can mimic a TTX poisoned axon simply by reducing (or abolishing) g_{Na}.You can study the effects of hyperpolarization and find that at more negative resting potentials, there are more Na^+ channels available for a quick response because they are fewer *h* gates blocking them.

Steady state plots show where the gates are 'heading'

In a voltage clamp experiment V_m is held constant by an external source of charge (see Voltage Clamp below for details). This means that all the *a*'s and *b*'s are also constant during the clamp, making *m, n*, and *h* linear. If the membrane potential is suddenly changed from any value (say V_r) and clamped to a new potential, V_m, then *m, n,* and *h* rise (or fall) to a new steady state evaluated at V_m as: .

Equation 21

$$\left.\begin{aligned} n_{Final} &= \frac{a_n}{a_n + b_n} \\ m_{Final} &= \frac{a_m}{a_m + b_m} \\ h_{Final} &= \frac{a_h}{a_h + b_h} \end{aligned}\right\} \; at\; V_m = V$$

Plots of Equation 21 in Figure 8 show where each of the variables *m, n*, and *h* are 'heading' at each V_m. Upon depolarization (increasing V_m) , *m* and *n* increase tending to open Na^+ and K^+ gates, while *h* decreases tending to close Na^+ gates. Further, at rest when $V_m = V_r \approx -65$ mV, we see that only a few *m* gates (5%) are in the open position, while a large number of *h* gates (60%) are open. (The percentage of open Na^+ *channels* is given by $m^3h = 0.05^3 \times 0.60 \times 100 = 0.0075\%$.).

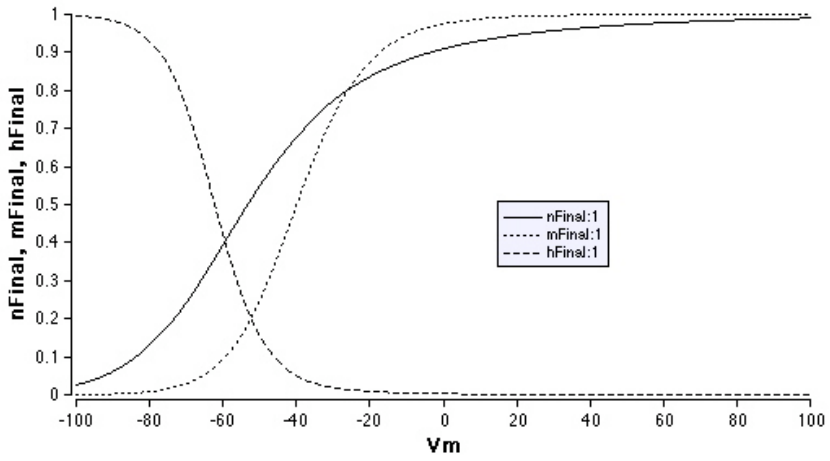

Figure 8. Plots of Equation 21 showing steady states of *m, n,* and *h* as a function of membrane voltage, V_m.

Relaxation time plots show how fast things are changing

The rates at which *m,n,* and *h* approach their steady state values can be summarized by plotting their respective relaxation times, T_m, T_h, and T_n which are computed as:

Equation 22

$$\left.\begin{aligned} T_m &= \frac{1}{a_m + b_m} \\ T_h &= \frac{1}{a_h + b_h} \\ T_n &= \frac{1}{a_n + b_n} \end{aligned}\right\} \quad at\ V_m = V$$

The plots are shown in Figure 9. We see that the *m* gates are much faster (smaller relaxation time) than either *h* or *n*. This is particularly true at potentials near the resting potential (-65 mV) and within the normal range of depolarizations that will trigger excitation. These results justify calling *m* gates fast, in comparison with *n* and *h* gates, which are called slow.

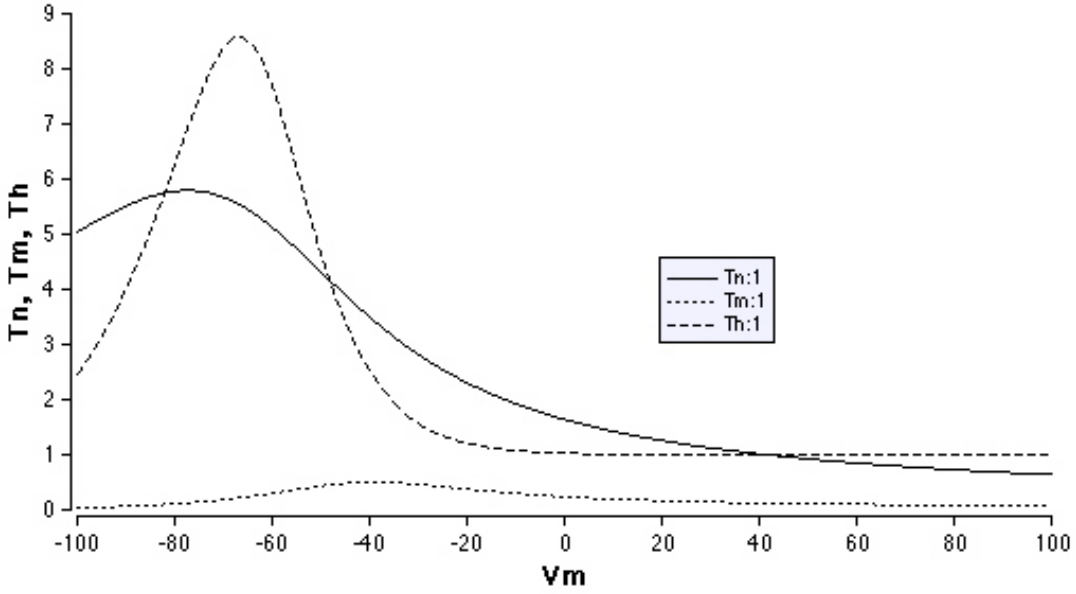

Figure 9. Plots of Equation 21 showing relaxation times of *m, n*,and *h* as a function of $V_{m()}$

Excitation has three phases

Recall that the K^+ concentration inside of the axon is high, while the outside concentration of Na^+ is high. Further, recall that the membrane potential is a measure of the electrical force on a positive charge. Finally, remember that the amount of charge movement necessary to make substantial changes in V_m is very small. During the short time of a single action potential, the actual amounts of Na^+ and K^+ that move in or out of the axon are very small; they have significant effects on V_m, but the concentrations of Na^+ and K^+ hardly change. At rest the axon is permeable mostly to K^+, but not much K^+ leaks out because the opposing membrane potential, V_m, is close to the K^+ equilibrium potential (i.e. the concentration gradient of K^+ is almost balanced by V_m pushing in the opposite direction (see Figure 12).

At rest, the membrane is polarized. At $V_m = V_r$, Figure 8 shows a number of slow (*h, n*) gates are open but most rapid *m* gates are closed so that most Na^+ channels are closed. When the membrane is stimulated, its response to the depolarization can be divided into 3 time phases:

1. **Early response** when the rapid m-gates open quickly. Now both *m* and *h* gates are open. The slow h gates have not had time to close in response to the depolarization. As a result, channels are freely permeable to Na^+, and Na^+ rushes into the axon causing V_m to rise in the positive direction.

2. **Late response.** A moment later the slow *h*-gate closes**.** The membrane is no longer highly permeable to Na^+, so the rapid inflow of Na^+ ceases. In addition, the slowly responding n-gates in the K^+ channel open and K^+ flows out of the axon, causing V_m to fall in the negative direction.

3. **Recovery phase**. Still later, the slow gates return to their original resting position.

The threshold occurs when Na^+ flowing in surpasses K^+ flowing out

A sub-threshold stimulus does not stimulate a sufficient Na^+ flow influx to overcome the outflow of K^+ and the axon repolarizes without firing. A supra-threshold stimulus triggers enough Na^+ channels to open so that Na^+ inflow exceeds K^+ outflow, and the *net* charge flow is now positive inward, But, this depolarizes the axon even further, and opens even more Na^+ channels which causes still more depolarization. A vicious cycle (positive feedback) ensues; the membrane potential takes off in the positive direction with an explosive velocity as the interior of the axon becomes more and more positive. But this rapid upward movement of V_m does not persist. Soon V_m becomes positive and large enough to oppose Na^+ entry despite the open channels, i.e. V_m approaches the Na^+ equilibrium potential where the concentration gradient moving Na^+ inward is balanced by V_m pushing Na^+ out.

At the same time the delayed effects begin to appear. Na^+ channels close and voltage activated K^+ channels open allowing K^+ outflow that exceeds Na^+ inflow. The net flow of charge is now positive outward, and V_m plummets toward its resting value, overshoots momentarily, and settles closer to the K^+ equilibrium potential (−77 mV) because the voltage activated K^+ channels are still open making the membrane even more K^+ permeable than it was at rest. Finally the repolarized membrane closes the voltage activated K^+ channels and V_m returns to its resting value. The positive and negative feedback loops are illustrated in Figure 10.

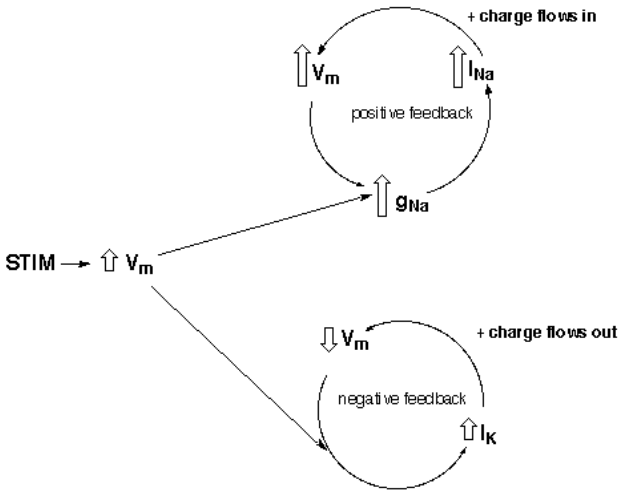

Figure 10 The threshold is where $I_K = I_{Na}$. With $I_{Na} < I_K$ the stabilizing negative feedback (lower cycle) dominates. When $I_{Na} > I_K$ the explosive positive feedback (upper cycle) dominates.

From this qualitative description, we see that the **firing threshold** is determined by the stimulus strength that is large enough to allow an inward Na^+ flow that exceeds the outward K^+ flow. From that point onward, the stimulus plays no further role because the ingredients of the positive feedback cycle reside in the axon itself. This is shown in Figure 11 where the absolute values of I_{Na} ($absI_{Na}$) and I_K are plotted together with the corresponding V_m for stimuli that are

(A) sub-threshold, (B) nearly threshold, and (C) supra threshold. The principal result is striking: no action potential develops until $absI_{Na} > I_K$. [2]

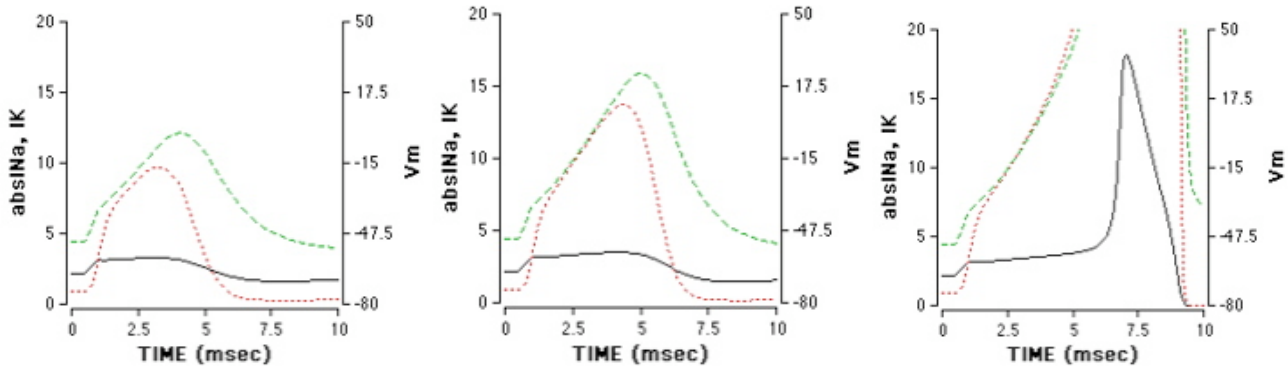

Figure 11 Plots of the absolute values of I_{Na} ($absI_{Na}$) and I_K together with the corresponding V_m for stimuli that are subthreshold (A with I_{stim} = 15.00), nearly threshold (B with I_{stim} = 15.275) and supra threshold (C with I_{stim} = 15.30).

V_m is limited by V_K and V_m

The **all-or-none** response arises naturally out of this positive feedback; once the response is triggered, the positive feedback drives the membrane potential to its maximum value (near the Na^+ equilibrium potential). The size of the action potential is determined, to a large extent, by the concentration gradients of Na^+ and K^+. The concentration gradient of K^+ limits the resting potential (K^+ equilibrium potential) while the concentration gradient of Na^+ limits the height of the action potential (Na^+ equilibrium potential). Just as a stick of dynamite contains its own explosive energy, the axon membrane is 'loaded' with 'explosive' energy in the form of ion gradients.

Although this description captures the essence of the excitation process, the precise position where excitation occurs is a bit more complicated because we have ignored contributions of the leak current and assumed that the process takes place before any *n* (K channels open) or *h* gates, (Na channels close) have time to develop. In addition, although the size of the action potential is limited by V_K and V_{Na}, it never reaches either one, and closer inspection (see Figure 25) reveals that axons do not follow all-or-non behavior as precisely as once held.

Refractory Period is determined by the return to steady state of slow gates, n, and h

For a brief msec or two following excitation, the axon is no longer excitable. This recovery phase is called the **refractory period**, during which the threshold appears to be infinite, followed by a gradual return to normal. The basis for the refractory period is found in the 'late effects'. After the first msec of excitation the slow Na^+ (*h*) gates close and remain closed for a brief time despite

[2] In some cases with stimuli that are just under threshold the $absI_{Na}$ may momentarily exceed I_K, only to fall back as the late response (K^+ channels open, Na^+ channels close) sets in.

the fact that V_m is near rest. These gates were slow to respond to the initial depolarization, and they are equally slow in responding to the repolarized membrane. In addition, the voltage activated K^+ gates are still open and this drives V_m *below* the resting potential, creating a dip called the **negative afterpotential**. With the slow Na^+ gates closed and the K^+ gates open, it is almost impossible for Na^+ inflow to exceed K^+ outflow in order to reach threshold. You can illustrate this by plotting the $absI_{Na}$ and I_K that result from a second stimulus delivered during the refractory period. To make this plot intelligible, you may have to use the Zoom button on the graph window to see the results.

In addition to Na^+ and K^+ channels, the axon is replete with Na^+–K^+ pumps. But do they influence the action potential? The answer is no, at least not directly. The ion fluxes driven by the Na^+–K^+ pump are swamped out by the more massive movements of the ions through the channels. The pump does not cycle often enough to make a measurable difference during an action potential. However, action potentials are very brief and the axon is at rest most of the time. At rest there is ample time for the slow cycling of the pump to restore the small amounts of Na^+ and K^+ that have leaked through channels activated during the action potential.

The Voltage Clamp uncouples V_m from the gates

Excitation of a normal axon involves the rapid, but transient increase in sodium conductance, g_{Na}, followed by a slower, more prolonged increase in potassium conductance, g_K. These changes in membrane permeability are governed by the opening and closing of ion channels. Study of the axon in its natural state is complex because the opening and closing of each channel gate depends on both time and V_m, and V_m in turn is changing in response to changes in the gates. This mutual interaction between gates and V_m endows the axon with excitation properties that allow it to respond in an explosive manner when stimulated.

To simplify analysis of the gates it is expedient to interrupt this cycle by making the membrane potential independent of the gates. This is accomplished by 'clampling' V_m at a constant preset level throughout the axon's response and then measuring the current (ion flow) through the channels while the measurement is underway. To find what portion of the current is due to Na^+, one can remove the Na^+, or poison the Na^+ channels; what current remains must be due to K^+—providing other ion leaks are negligible.

Voltage Clamp Experiment

For simplicity, we illustrate the voltage clamp experiment in an axon with poisoned Na^+ channels and ignoring the small leakage currents. In this case all current is carried by K^+.

At rest the concentration gradient driving K^+ diffusion out of the cell is balanced by membrane voltage acting in the opposite direction. Consequently, very little K^+ leaks out (see Figure 12).

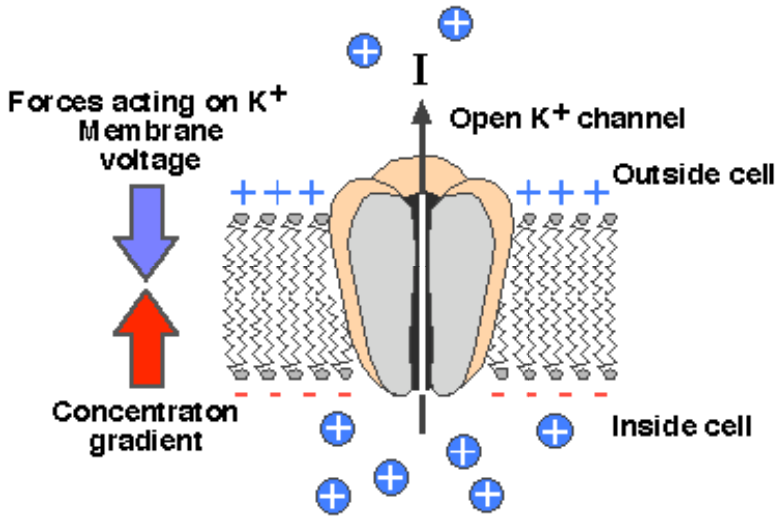

Figure 12. Forces acting on the K^+ ions before the clamp is turned on.

When the voltage clamp is turned on, a small pulse of negative charge is delivered to the external membrane surface and an equivalent positive charge is delivered to the internal surface. This new charge is just sufficient to jump the membrane potential from -65 mV to -20 mV (Figure 13a).

This new -20 mV membrane potential tending to force positive charge into the cell is too weak to balance the tendency of K^+ to diffuse out of the cell. In addition, the depolarization of the membrane opens more K^+ channels. K^+ diffusing out of the cell would add positive charge to the outside and change the membrane potential, but the voltage clamp monitors V_m and prevents any change by adding one negative charge for each K^+ that crosses the membrane out of the cell (Figure 13b).[3] Thus, the compensating current delivered by the external electrode is a precise measure of the K^+ leaving the cell. The value of the voltage clamp is due to the fact that it is not possible to chemically measure the small amounts of K^+ that enter or leave the cell within a fraction of a millisecond, but the *charge* delivered by the voltage clamp can be measured easily.

If both Na^+ and K^+ channels are open then the total measured voltage clamp current equals the sum of the individual Na^+ and K^+ currents. Subtracting the K^+current (measured as above in a separate experiment) from the total current leaves the Na^+current.

[3] The negative charge added to the solution is an ion, not a bare electron. The identity of the ion depends on the type of electrode and need not concern us here

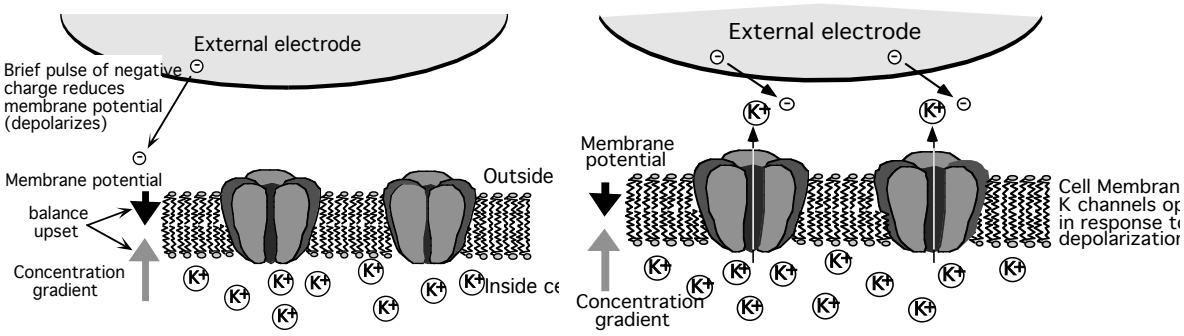

Figure 13. (a) The voltage clamp has been turned on, charging the membrane to a new pre-set potential. (We have not shown the intracellular electrode which acts in a similar way by 'absorbing' the excess negative charges (ions) left behind by K^+ when it moves to the outside.) (b) K channels open in response to depolarization. Charge delivered by the electrode compensates for the K moving out of the cell preventing any change in membrane potential. The current delivered by the electrode = I_K.

Building the Voltage Clamp

In this section we will lead you through the procedure for building a voltage clamp model that will impose a constant membrane potential on the model axon.

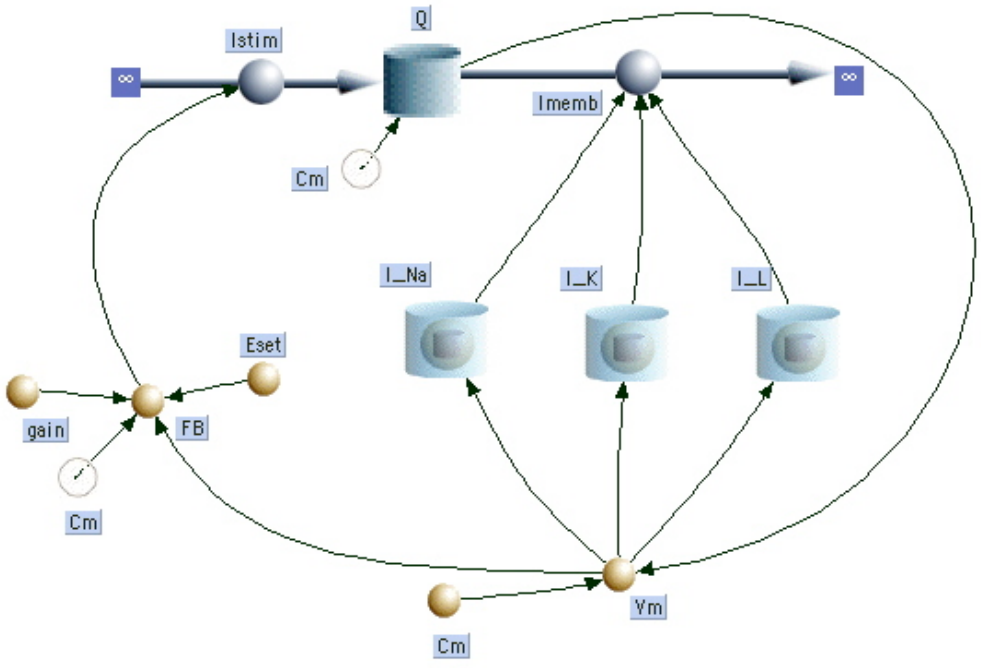

Figure 14 Flow chart for the voltage clamp model.

1. Choose a desired membrane potential; call this V_{set}.

2. Add charge to the membrane until the membrane potential is equal to the desired amount. This is accomplished by adding $(V_{set} - V_r)C_m$ to the membrane. Be careful with + and - signs on the V's, and remember: V_r is negative! One good way to set this up is to use the PULSE function with the desired charge as the 'volume' part of the pulse. For example, if you wanted to start the voltage clamp after 0.2 msec have elapsed, use the following stimulus:

$$Istim = CHARGING\ CURRENT = PULSE((V_{set} - V_r)*C_m, 0.2, 1000)$$

The electrical current containing this pulse of charge is called the 'charging current'. Although this will create the desired change by giving V_m a good boost to get it close to V_{set}, it will not hold it there because ions will immediately commence to move. To maintain V_m close to V_{set}, you will have to monitor V_m at all times and keep adding or taking away charge via the stimulating electrodes to stop V_m from drifting. This compensating current used to maintain V_m close to V_{set} is called the 'feedback current'.

3. Define a variable called **error** as the difference between V_m (the membrane potential) and V_{set}. Keep adding charge proportional to this error (via the stimulating electrodes) so that the error is always close to zero. If the error is positive, you will have to take + charge away from the internal membrane surface to compensate (i.e. inject a negative current), and vice versa. (Note that if you make a mistake in the sign of your current, you will change a negative feedback into a positive one, and the system will go unstable!) The proportionality constant that relates the feedback current to the error is called the '**gain'**.

$$FEEDBACK = -\ GAIN*(V_m - V_{set}) = -\ GAIN*(error)$$

Increasing the *GAIN* increases the sensitivity of the feedback: with a large *GAIN*, a small error will induce a large feedback response.

To clamp the voltage for a prolonged period of time, the feedback current should be added to the charging current (again via the stimulating electrodes). However, our feedback current is not used to create a new potential, but only to maintain it. Therefore it is best to add the feedback current just *after* the pulse has ceased.

To begin the clamp after 0.2 msec, charge up the membrane with a pulse starting at 0.2 msec and lasting for only DT additional msec. As soon as the pulse is complete, i.e. at 0.2+DT msec the feedback is turned on. We have

Equation 23 $I_{stim} = CHARGING\ CURRENT + FEEDBACK$

$$= PULSE\ ((V_{set} - V_r)*\ C_m\ , 0.2, 1000) - GAIN*(V_m - V_{set})*\ STEP(1, 0.2+DT)$$

The feedback current is easily measured, but what does it tell us? Since the voltage is held constant, the membrane charge cannot be changing. Therefore, the only time we can add a charge from our electrodes to the inner surface of the membrane is when a charge of like sign has left this surface. In other words, *the feedback current measures the net charge flowing through the channels*. It is just what we need to compute the opening and closing of channels, since, if the current is large, there must be many channels open. To find what portion of the

current is due to Na^+, subtract the Na^+ current (or poison the Na^+ channels); what remains must be due to K^+ and leaks.

4. Try V_{set} = –20 mV and experiment with different values of *GAIN* to see how close you can hold (clamp) the membrane potential to –20 mV. Set the duration at 8 msec.

5. Record both V_m and I_{stim}. The feedback of I_{stim} is a measure of the net charge flowing through the channels.

6. Compare the magnitude of I_{stim} in the voltage clamp measurements with the same quantity in the excitation experiments. This gives some idea of the magnitude of the relatively small stimulus (charging current necessary to depolarize the axon to some threshold value) and the huge response of the intact nerve (charge flowing through channels).

7. The steady state error is given by the deviation of V_m from V_{set}. This quantity is important in feedback systems. Can you reduce it by increasing the *GAIN*? Show that if the *GAIN* is too small V_m is not clamped, but if the *GAIN* is too large the system is unstable. This instability is due to an inevitable time lag between the *error* detection and the feedback signal.

8. Separate the contributions of Na^+ and K^+ to I_{stim}. You can accomplish this by recording I_{stim} after poisoning (removing) one channel type and studying the other. To a good approximation, you can ignore the contribution of the leakage current, I_L. Verify this by plotting the leakage current on the same scale as I_{stim}. Note that both I_{Na} and I_K have a measurable delay before changing at their maximal rate. This is not characteristic of a simple exponential (linear) process, and it is what prompted Hodgkin and Huxley to consider raising n and m to a power (i.e. n^4 and m^3). Try measuring I_{Na}, and I_K at several different voltages (V_{set}).

9. Try changing the system into a positive feedback—you will see the system go unstable very quickly!

The Hodgkin-Huxley Model Updated

Although the Hodgkin Huxley (HH) model accounts for an impressive array of experimental data, like any theory it has its limitations and inaccuracies. In particular, data obtained since the original HH model was proposed raise the following issues:

- The HH model assumes that the ionic currents are linear functions of membrane voltage, V_m. Although the authors supplied evidence that this was a reasonable assumption, more recent experiments show a non-linear relation that can be accounted for by the Goldman-Hodgkin-Katz (GHK) description of ion diffusion in a constant electric field (see the Appendix). Further, the HH model was devised to describe the squid axon under a narrowly defined set of conditions: the linear relation does not have a built-in mechanism for incorporating the dependence of g_{Na} or g_K on ion concentrations, but the GHK equations do.

- The HH model is inconsistent with single ion channel recordings (patch-clamps) and with measurements of very small currents (gating currents) that reflect charge movements associated with conformational changes in channel proteins that occur during excitation. This data provides strong evidence that, contrary to HH model, inactivation (*h* gates) and activation (*m* gates) of the Na channel are not independent and that the channels move through a sequence of dependent states before opening (Hille 2001; Patlak 1991).

- The rate constants in the HH are empirical functions of voltage. Since there is no ready means to interpret them, they are a dead end in the quest for molecular mechanisms underlying ion channel physiology.

- In addition to the above there are a number of experimental discrepancies that require extensions, including the response to sustained stimuli, the voltage dependent block of Na channels by divalent cations, and changes of external ion concentrations as they are trapped within the small spaces between the axon and supporting glial cells (Clay 1998).

An updated squid axon model (Clay 1998) that addresses these issues follows.

A revised Na^+ current model uses the GHK equations plus a new channel model

Our updated version replaces the original Na current submodel (Figure 3) with the submodel shown in Figure 15.

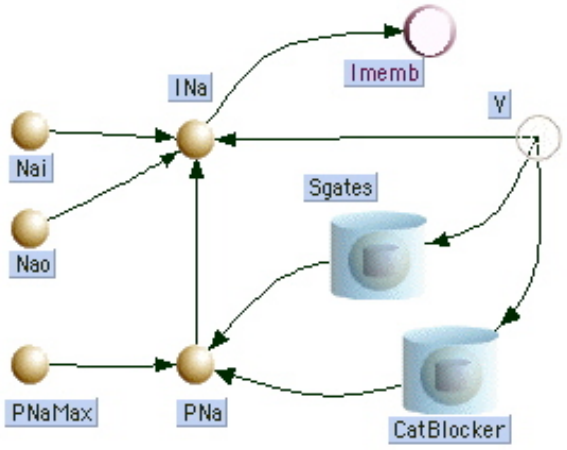

Figure 15 Updated I_{Na} submodel. Replacement of both m and h gates are contained within the Sgates sub model. The CatBlocker submodel contains the influence of divalent cations on the Na^+ channel. g_{Na} is replaced by P_{Na} and the dependence of I_{Na} on Na^+ concentrations is now explicitly taken into account by the GHK flux given in Equation 26.

The object of the submodel is to compute the Na^+ current from the GHK equation under all conditions. We begin with a simpler case where all channels are open and we replace the Hodgkin-Huxley expression for the maximum Na^+ current. Thus, $I_{NaMax} = g_{NaMax}(V_m - V_{eq})$, is replaced by the constant field equation (see the Appendix for derivation):

Equation 24

$$I_{NaMax} = \frac{P_{NaMax}\dfrac{FV_m}{RT}}{e^{\frac{F}{RT}V_m} - 1}\left(Na_i e^{\frac{F}{RT}V_m} - Na_o\right)$$

where P_{anama} [cm·sec^{-1}] represents the permeability coefficient of the membrane with all channels open, while Na_i and Na_o [mM·L^{-1}] represent the concentration of Na in the internal and external solution (see the Appendix for resolution of units)

Normally, only a fraction of the available channels are open. There are two independent sources for this attenuation: (1) Many channels are in a closed configuration, and (2) divalent cations in the external solution can block channels. Let S_6 denote the fraction of channels that are in the open configuration, and f_b denote the fraction that are blocked by divalent cations. Then the fraction that are not blocked is given by $1–f_b$, and the fraction of channels that are both open and not blocked by cations is given by $S_6(1-f_b)$. We use this result to define a dynamic Na^+ permeability, P_{Na}, by

Equation 25

$$P_{Na} = S_6\left(1 - f_b\right)P_{NaMax}$$

An expression for the Na current that applies to all cases is obtained by substituting P_{Na} for P_{NaMax} i.e.

Equation 26

$$I_{Na} = F\frac{P_{Na}\dfrac{FV_m}{RT}}{e^{\frac{F}{RT}V_m} - 1}\left(Na_i e^{\frac{F}{RT}V_m} - Na_o\right)$$

S_6 and f_b are computed in the two subprograms (Sgates and CatBlocker) indicated in Figure 15 and described in the following paragraphs.

Revised Na Channel model uses 9 configurations to compute S_6

The channel model depicted in Figure 16 and Figure 17 assumes that channel proteins pass through a series of five conformations, *S1… S5*, before arriving at a state that can lead to an open channel, *S6*. These replace the *m* gates of HH. The *h* gate is replaced by three inactivation states *SI4 , SI5* and *SI6* as indicated. The variables *S1 … S6*, or *SI4...SI6* represent the probability of finding a channel in their respective state

As shown below and in Table 4, all rate constants involved in the transitions between channel states are exponential functions of voltage. Those illustrated in red increase with depolarization (stimulation), those in black decrease with depolarization. The states *S1… S5* are intermediate conformations leading directly to the open channel configuration. All forward transitions in this sequence (the a's) are enhanced by depolarization driving the channel to S6, the open configuration. At first sight the *S6,* the states *SI4… SI6* are simply members of a detour to bypass *S5*. However, once a channel is in any of these closed states the only escape is via transition bI_4 or aI_6. But both of these transitions (colored black in Figure 16) decrease upon depolarization. Moreover this decrease is not trivial. For example, inspection of Table 4 shows that when V = 0 both bI_4 and aI_6 are two orders of magnitude smaller than any of the other rate constants. Upon depolarization, the *SI4… SI6* configurations trap the channel in a closed,

inactive form. This is analogous to the closure of h gates on depolarization in the original HH theory. Hence the *SI4… SI6* configurations are referred to as *inactivated* states.

Transitions between sites are all reversible and the rate constants governing these transitions are modeled using absolute rate theory.

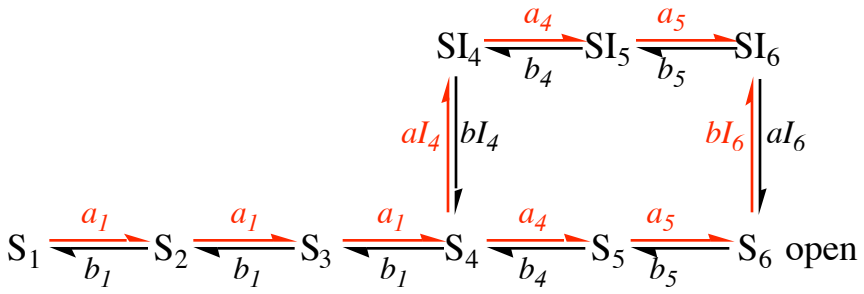

Figure 16 Schematic of updated Na^+ channel model (Vandenberg and Bezanilla 1991). S_6 is an open channel state. All other states are closed. Forward rates (i.e. those leading to S6) are designated by *a* while the reverse rates are represented by *b.* Rates that increase on stimulation (depolarization) are shown in red, rates shown in black decrease with stimulation. Note that all rates leading to S6 increase with the exception of *aI6* which decreases.

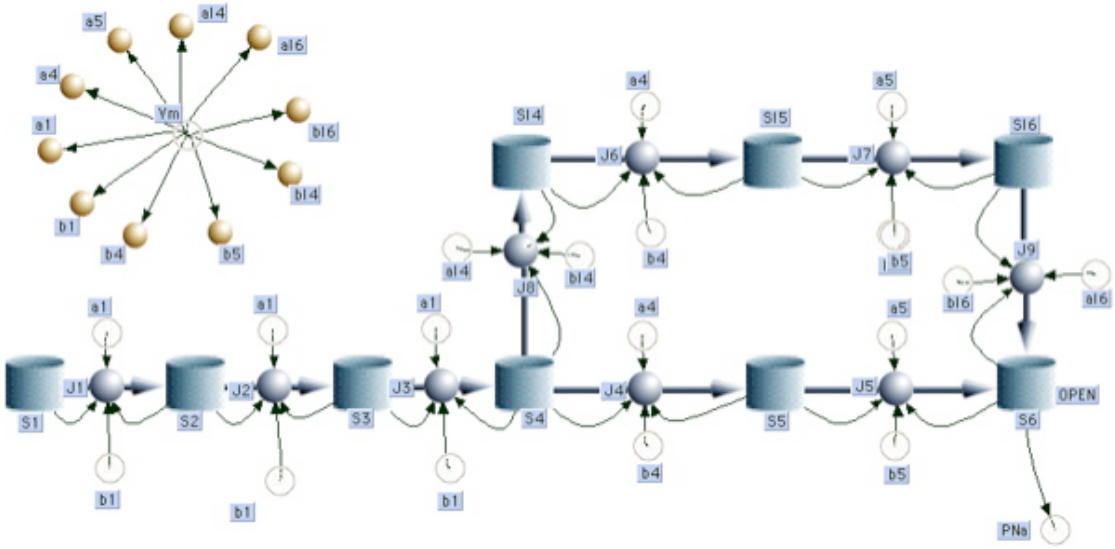

Figure 17 Madonna Flow Chart representation of updated Na channel model. To avoid clutter, the voltage dependent rate constants are computed in the upper left circular diagram, and aliases of rate constants are employed in the rest. Note that all dynamic icons are simple repeating units. The subtle behavior of the channel is due to the voltage dependence of the different rate constants.

Transition rate constants are obtained from absolute rate theory

The formalism of absolute rate theory, is often used to model transport processes as well as chemical reactions (Berg, Tymoczko and Stryer 2002; Weiss 1996). In passing from one state to another, say from *S2* to *S3*, an activation energy barrier $\Delta G^{\ddagger}$ must be surmounted. The rate constant, *k*, is proportional to the fraction of molecules having the requisite energy, and accordingly is set equal to $k_o \exp(-\Delta G^{\ddagger}/RT)$ where k_o is a constant of proportionality. The exponential can be viewed as the probability of surmounting the energy barrier, and k_0 as the frequency to attempts per unit time. $\Delta G^{\ddagger}$ represents the work done in moving from a local energy minima to the next peak (see Figure 18). This work can be split into the sum of two parts , $W + W_e$, where W_e denotes the electrical work done by movement of the gating charge (i.e. electrical charge moving with the conformational change), and all other work is denoted by *W*.

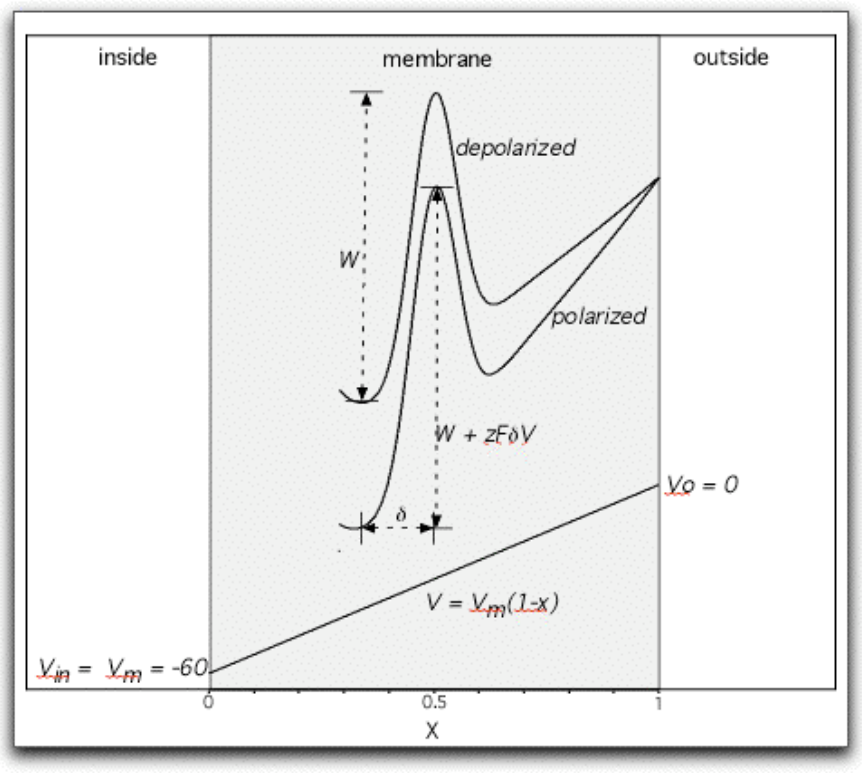

Figure 18 Energy barrier interpretation of rate constants.

If z denotes the charge moved and V_v is the electrical potential at the local minimum (valley) with V_p its value at the maximum (peak), then by definition of electrical potential, $We = zF(V_p - V_v)$. Assuming the voltage drop through the membrane is linear (constant field), and letting *x* denote the fractional distance from the inside membrane (i.e. *x* is the actual distance divided by the membrane thickness) we have

Equation 27 $$V = V_m(1 - x) \qquad 0 \le x \le 1$$

where it is assumed that the external solution is grounded, making $V_o = 0$. If d_p and d_v denote the x coordinates at the peak and valley respectively, then $V_p = V_m(1 - d_p)$ while $V_v = V_m(1 - d_v)$, so that $V_p - V_v = -V_m(d_p - d_v)$. Defining $\delta = d_p - d_v$ as the distance between peak and valley we have, $We = -zFV_m\delta$, and it follows that the voltage dependence of the rate constant is given by

Equation 28

$$k = k_o \exp\left(-\frac{\Delta G^{\ddagger}}{RT}\right) = k_o \exp\left(-\frac{W + W_e}{RT}\right) = k_o \exp\left(-\frac{W - \delta zFV_m}{RT}\right) = k_p \exp\left(\frac{\delta zFV_m}{RT}\right)$$

where $kp = ko \cdot exp(-W/RT)$. Numerical values for the rate constants for the model in Figure 17 are listed in Table 4

The flows are given by:

Equation 29

$$\begin{cases} J1 = a_1 S_1 - b_1 S_2 \\ J2 = a_1 S_2 - b_1 S_3 \\ J3 = a_1 S_3 - b_1 S_4 \\ J4 = a_4 S_4 - b_4 S_5 \\ J5 = a_5 S_5 - b_5 S_6 \\ J6 = a_4 SI_4 - b_4 SI_5 \\ J7 = a_5 SI_5 - b_5 SI_6 \\ J8 = aI_4 S_4 - bI_4 SI_4 \\ J9 = aI_6 SI_6 - bI_6 S_6 \end{cases}$$

The Na Channel is blocked by Divalent Cations

Channel blockage can be addressed by assuming the cations react with binding (receptor) sites on the channel. Letting f_b represent the fraction of sites that are blocked by cations we use EQUATION XXX. Pharmacol Chapter to write

Equation 30

$$f_b = \frac{C'_c}{K_{50} + C'_c}$$

Where C'_c is the divalent cation concentration at the site while K_{50} is the dissociation constant. The site concentration, in equilibrium with the corresponding external concentration, $C_{c,}$, is obtained by Equation 46 as $C'_c = C_c \exp(2*FV'/RT)$ where V' is the potential at the binding site. But, letting d_c represent the x coordinate of the binding site we have, from Equation 27, $V' = V_m(1-d_c)$ so that

Equation 31

$$f_b = \frac{C'_c}{K_{50} + C'_c \exp\left(-\frac{2F}{RT} V_m (1 - d_c)\right)}$$

The *CatBlocker* submodel is illustrated below.

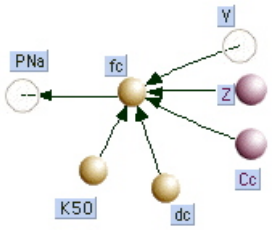

Figure 19 Submodel for *CatBlocker*

K Current is described by the GHK equations

Although 'State' models similar to the Na channel model, illustrated in Figure 15, have been proposed (Destexhe and Huguenard 2001; Perozo and Bezanilla 1990) for K channels, we follow the Clay revision that retains the fourth order, independent gating, scheme of Hodgkin-Huxley. However the equation for open channel current flow is replaced with the constant field (GHK) assumption and the functioning K current is replaced by

Equation 32

$$I_K = Fn^4 P_K \frac{\frac{FV_m}{RT}}{e^{\frac{FV_m}{RT}} - 1}\left(K_i e^{\frac{FV_m}{RT}} - K_s\right)$$

where F is the Faraday constant, K_s represents the external K concentration just adjacent to the channel and n has the same meaning as in Figure 6.

K efflux is trapped in the axon - glial space

In the original HH theory it was assumed that the large volume of the external solution insured that K_s changes are insignificant so that it could be assumed constant. More detailed studies of a series of excitations show that the descent of the final portions of successive action potentials becomes less and less with each action potential, suggestive of a rise in K_s. A structural basis for K_s accumulation is provided by the small spaces between the axon membrane and supporting glial cells that adhere to the axon. This can be incorporated in the model by letting K_s be a variable as shown below.

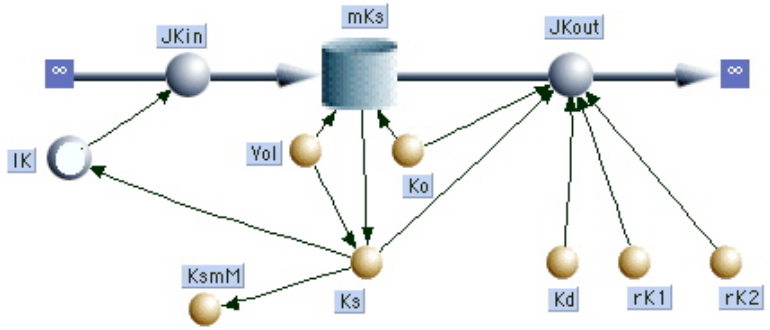

Figure 20 Submodel for K accumulation in the 'glial-axon space' *KTrap* of Figure 21

In Figure 20, *mKs,* the milimoles of *Ks* within the glial-axon space , is increased by I_K as

Equation 33 $$JK_{in} = 1.e\text{-}6 * \frac{I_K}{96485}$$

The numerical factors arise because I_K is given in nCoul·msec^{-1} = 10^{-9} Coul·msec^{-1}. Dividing I_K by the Faraday constant 96485 converts it 10^{-9} mol ·msec^{-1}, and multiplying by 1000 converts to 10^{-6} mmol · msec^{-1}.

Dissipation of *mKs,* is given by the sum of two processes:

Equation 34 $$JK_{out} = r_{K1}(K_s - K_o) + r_{K2}\frac{(K_s - K_o)}{(1 + (K_s - K_o)/K_d)^3}$$

where r_{K1}, r_{K2}, and K_d are constants. The first term accounts for simple diffusion from the glial-axon space to the bathing medium while the second term refers to a postulated, but unspecified glial cell uptake of excess K^+ form this space. The second term is essentially an empirical expression that describes observed K^+ movement out of the space. K_S is obtained in proper units (mM) for use in Equation 32 if *mKs*[mmol] is divided by *Vol*[L].

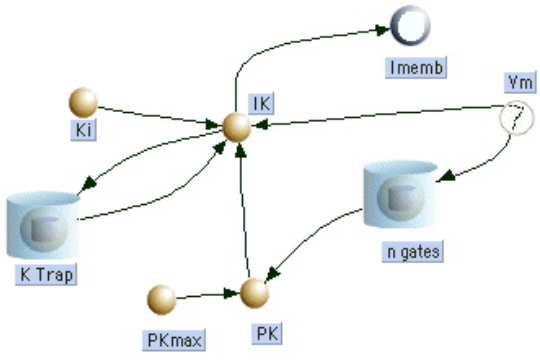

Figure 21 Submodel for IK

Build the Model

Modify the basic HH model using the flow charts illustrated in Figure 15, Figure 17, Figure 19, Figure 20, and Figure 21 together with Equation 25, Equation 26, Equation 29, Equation 31, Equation 32, Equation 33, and Equation 34, as well as the parameters listed in Table 4

Table 4 Parameters for updated axon model

T	8 degrees C
F/RT	1/24 mV^{-1} at 8 degrees C

Sodium

Na_i	30 mM
Na_o	430 mM
P_{NaMax}	$1.24 \cdot 10^{-4}$ cm·sec^{-1}
$a1$	19.1 exp(0.014 V) sec^{-1}
$b1$	2.04 exp(-0.048 V) sec^{-1}
$a4$	6.37 exp(0.017 V) sec^{-1}
$b4$	5.61 exp(-0.00017 V) sec^{-1}
$aI4$	1.00 exp(0.00004 V) sec^{-1}
$bI4$	0.0132 exp(-0.038 V) sec^{-1}
$a5$	11.5 exp(0.06 V) sec^{-1}
$b5$	2.20 exp(-0.02 V) sec^{-1}
$aI6$	0.00760 exp(-0.038 V) sec^{-1}
$bI6$	0.560 exp(0.00004 V) sec^{-1}
C_c	60 mM
K_{50}	150 mM
d_c	0.81

Potassium

K_i	300 mM
K_o	10 mM
P_{Kmax}	$5.18 \cdot 10^{-5}$ cm·sec^{-1}
a_n	0.01*(V+55)/(1-exp(-(V+55)/10))
b_n =	0.10*exp(-(V+60)/25)
init Ks=	$Vol \cdot K_o$
r_{k1} =	1.67E-7
r_{k2} =	1E-5
K_d =	2mM
Vol =	1.1E-6 L

Steady state initial conditions can be found by running the model from arbitrary starting points.

All initial conditions are fairly explicit except those for the *S* states. These need to be determined from the steady state. There are two methods for finding steady initial conditions . The first and most straight forward is to set all flows equal to zero and solve the resulting simultaneous equations. We did this for *m*, *n*, and *h* in the original HH model. In the revised case, for the *S* states we would set all *J*'s in Equation 29 equal to zero and solve for the channel states. Often these equations cannot be solved analytically and we resort to numerical solutions. (Berkeley Madonna has a routine for this called *RootI* – see the menu item *Help>Equation Help* scroll to *Root Finder Equations*). Unfortunately, with a complex set of equations this is not always feasible.

The alternative, workable, but less elegant, method that we pursue here is to let the model find its own steady state. We guess values for all the unknown initial conditions start the model running with no stimulation throughout, and keep it running until it settles into a steady state. Values in this state will be valid initial conditions for the next simulation that will include the stimuli or conditions that characterize your specific problem. In practice it is simpler to carry out the procedure within a single simulation by running it for a long time, and then applying the stimuli near the end. In our case, suppose we are interested in the wave form of a single action potential over a period of say 10 msec. Set the *stoptime* = 110 msec and initiate the stimulus at 100 msec. The system will have 100 msec to settle into a steady state and you can zoom into the last 10 msec for your record. Of course you will also want to examine the first 100 msec to verify that the system has in fact settled into a steady state (more time may be needed). Once you have established a practical time for the system to settle down you may want to set the time axis on

the graph so that it will only begin plotting where your record begins (e.g. at 100msec). To do this choose the menu item *Graph >axis settings…*, uncheck *Auto*, and change the initial point on the *x axis*.

For example A crude set of initial guesses would set all S gates initially equal to 0.111 (There are 9 gates and by definition the sum of all S values =1). Run the model with these initial values (and no stimulation until the S values flatten out to a constant value, Use the new (steady state) values as the initial guess in subsequent runs where you allow stimulation and/or other changing conditions.

Compare an observed action potential with the HH and revised model predictions.

Figure 22 shows a recorded action potential obtained with a 40μA cm $^{-2}$ pulse 0f 1 msec duration contrasted with the HH prediction. Note that the minimum (arrow) in the experimental trace occurs several msec later than predicted by the HH model. Verify that the revised model can reproduce this delayed minimum by setting: *Intensity* = 40μA cm $^{-2}$, *Stimulus* = *Intensity* ·*SquarePulse*(5,1), V_r = - 63 mV, and *stoptime* = 25 msec

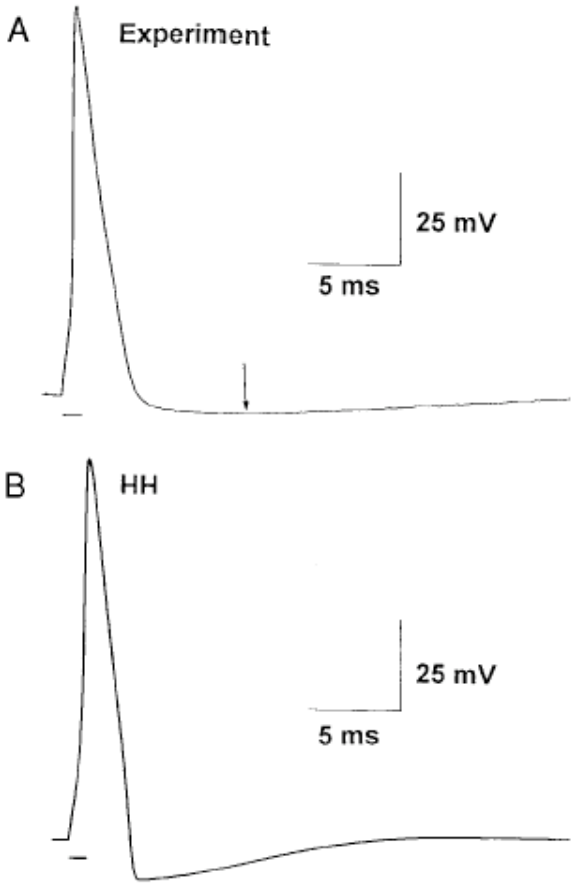

Figure 22 A. Action potential obtained with a 40μA cm $^{-2}$ pulse 0f 1 msec duration. The arrow points to the minimum in the recording. B, HH prediction with same stimulus (Clay 1998).

Show that the observed delay is due to K accumulation. You can study effects of K accumulation by changing the Vol, the axon-glial interspace volume. The larger the volume, the smaller the accumulation.

Revised model predicts response to sustained stimulus

Figure 23 shows a recorded action potential obtained with a 30μA cm $^{-2}$ stimulus lasting 60 msec together with the corresponding HH prediction. HH predicts a train of impulses following the first, while the experimental record shows a quiescent period. Using the same stimulus parameters (30μA cm $^{-2}$ for 60 msec), show that the revised model reproduces the experimental results.

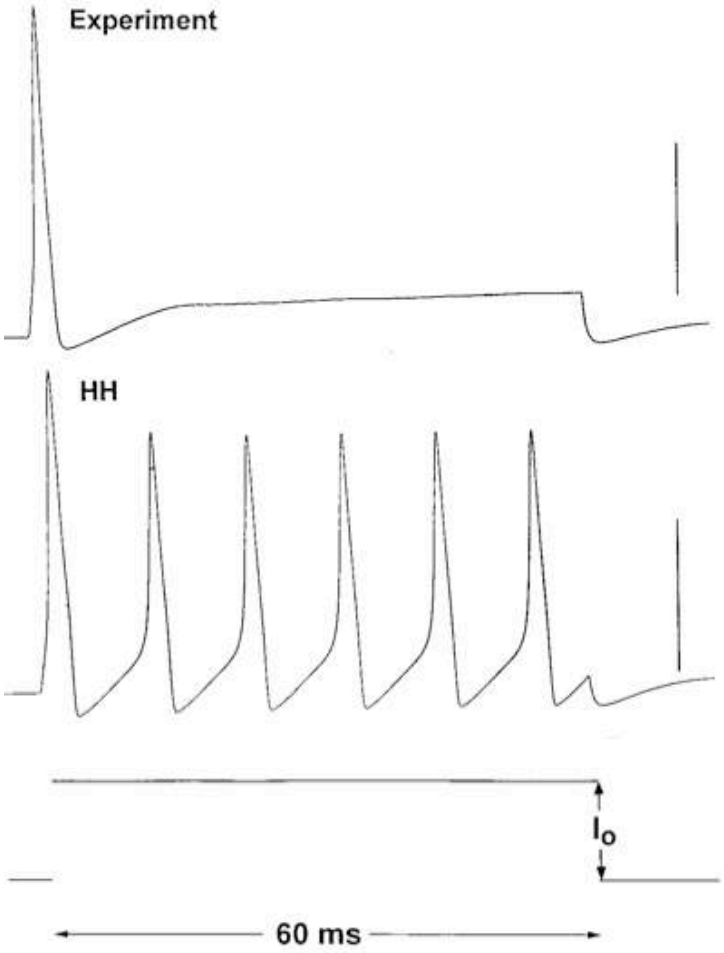

Figure 23 Response to sustained 30μA cm $^{-2}$ stimulus lasting 60 msec. The vertical calibration bar corresponds to 50 mV(Clay 1998).

Revised model predicts response to train of low intensity pulses

Figure 24 shows the recorded response to a repetitive set of weak stimuli with intensity = 10μA cm $^{-2}$. Each stimulus pulse lasted 1 msec with The interval between stimuli was 9.5 msec, while the duration of each stimulus was 1 msec. Note that HH predicts a response to every other stimulus, while the experimental record, again, shows a quiescent period. To test the revised model you will need to reproduce the periodic stimulus pattern.

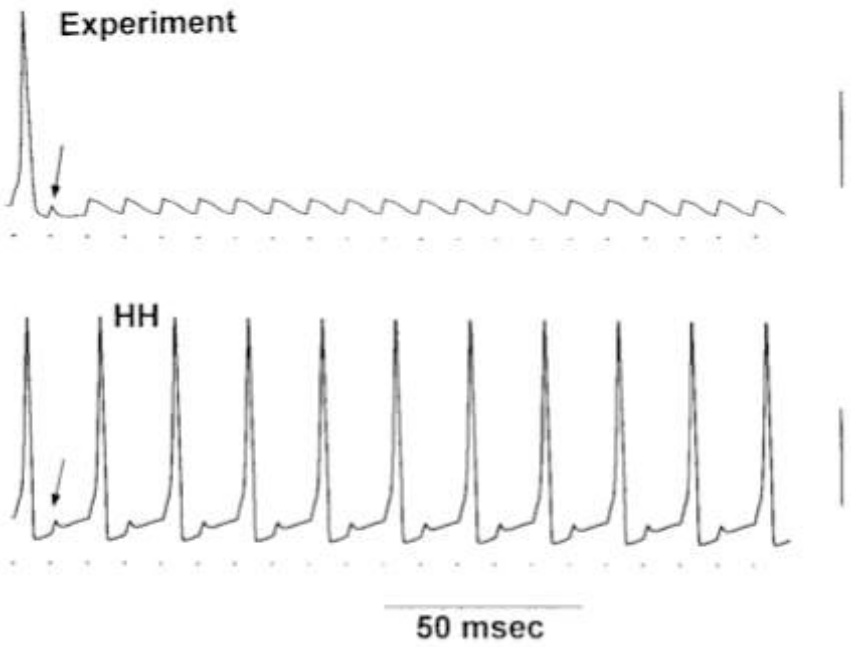

Figure 24 Response to a periodic series of pulse stimuli. Top: Experimental record obtained with 10 μamp/cm^2 pulses, each lasting 1 msec. The time interval between the 1 msec pulses was 9,5 msec. Bottom: HH prediction. (Clay 1998).

Use the mod function to create a periodic time base for periodic functions.

To make any arbitrary function, *f(t)* repeat itself every *Tp* time units we insert a new time clock, *tc*, within the function that resets itself to 0 each time it reaches *Tp*. In Madonna *tc* is given by

Equation 35 $$tc = \mathrm{mod}(time, Tp)$$

To make *f(t)* periodic we replace *t* with *tc*. For example, to produce a periodic train of exponential decays of the form *f(time)*= exp*(-k*time)*, we simply write Equation 35 and then use exp*(-k*tc)*. To create our repetitive stimulation pattern, we cannot use the *squarepulse* function for *f(time)* because it is not an explicit function of *time*, However, note that a single stimulation delivered at 9.5 msec can be written as *f(time)* = IF *time*< 9.5 THEN 0 ELSE *intensity*. If the same function is repeated every 10.5 msec (*Tp* = 10.5), then the stimulus will last for 1 msec. The stimulus pattern is implemented by replacing *time* with *tc*. We use Equation 35 together with

Equation 36 $Istim = \text{IF } tc < 9.5 \text{ THEN } 0 \text{ ELSE } intensity$

Find the threshold intensity for a 1 msec stimulus. Run the revised model at this threshold intensity for the pulse train defined by Equation 36 and try to reproduce the main features of the experimental results shown in Figure 24. Show that these results are not due to accumulated K.

The all-or-none response is not observed with stimuli close to threshold.

Figure 25 shows the recorded response to a set of four, weak, 1 msec, stimuli with different intensities near threshold. Three of the stimuli elicit action potentials, while the fourth is barely below threshold and does not evoke any active response. Results are superimposed for ease of comparison. Notice that the experimental results shows a definite gradation in the height of the action potential; the weakest stimulus gives rise to the longest delay as well as the smallest potential. Corresponding HH results shows similar delays, but the heights of the three action potentials are virtually equal. Stimuli used in the original record were threshold +1.0 and threshold ± 0.1 $\mu A\ cm^{-2}$. Using similar stimuli show that the revised model can replicate the main features of the experimental recording. Use the *Overlay* button to superimpose your results.

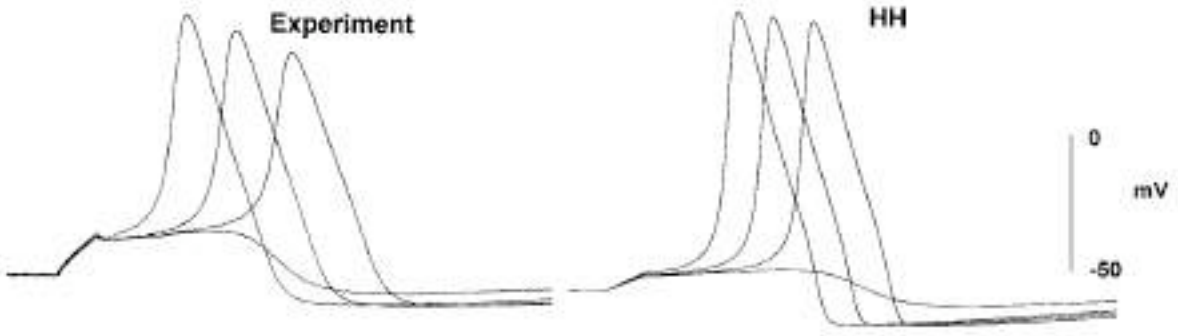

Figure 25 Left: Action potentials generated by 4, near threshold,1 msec stimuli. threshold +1.0 and threshold ± 0.1 $\mu A\ cm^{-2}$. Right: HH prediction. (Clay 1998).

These few examples show that the revised model is, indeed more accurate than the original HH formulation. However, it's real significance is that unlike the orthodox HH model it simulates channel behavior on a molecular scale and lends itself to interpretation in terms of molecular structure.

Projects

1 Model the temperature dependence of Squid axon excitability.

Hodgkin and Huxley measured the temperature dependence of the rate constants in their model (the *a*'s and the *b*'s) and found that each had a $Q_{10} \approx 3$. By definition the Q_{10} is the relative increase in the rate when the temperature is raised 10° C. If $a(T_0)$ is a known rate constant at temperature = T_0 then the value of *a* at any temperature *T*, will be given by

Equation 37 $$a(T) = a(T_0)Q_{10}^{(T-T_o)/10}$$

In the HH model values of the rate constants were determined at 6.3°C. So T_0= 6.3 while $Q_{10} \approx$ 3. Use these values to apply a temperature correction (similar to Equation 37) to the a's and b's of the HH model.

Compare the action potentials at 6.3°(common squid environmental temperature), 20°(room temperature), and 37°(mammalian body temperature) by super imposing them on the same graph. Note the speed of response as well as the height of the action potential. How does the threshold vary with temperature

Can you account for these differences? Verify your explanation. E.g. examine various currents? Plot steady state and relaxation time curves similar to those of Figure 8 and Figure 9 at the three temperatures.

2 Nodes of Ranvier at 37°C: voltage activated K channels are absent.

The nodes of Ranvier of mammalian myelinated axons have do not have voltage activated K**.** Following the peak of the action potential, the nerve relies on a strong leakage current to return the membrane potential to rest. The CRRSS (ref) model simulates these nodes in rabbits by making the following modifications in the HH model (units are the same as in HH):

$C_m = 2.5$	$g_L = 128$	$g_{NaMax} = 1445$	$\underline{\underline{I_K = 0}}$
$V_r = -80$	$V_L = -80.01$	$V_{Na} = 35.64$	
Temp = 37		$g_{Na} = g_{NaMax} m^2 h$	
$I_{memb} = I_L + I_{Na}$	$I_L = g_L*(V_m - V_L)$	$I_{Na} = g_{Na}(V_m - V_{Na})$	

$a_m = (126 + .363V_m)/(1 + \exp(-(V_m+49)/53))$ $a_h = b_h / \exp((V_m+74.5)/5)$

$b_m = a_m / \exp((Vm+56.2)/4.17)$ $b_h = (15.6)/(1 + \exp(-(V_m+56)/10))$

Implement this model and run it. Note that g_{Na} now depends on m^2h rather than the original m^3h. This action potential is very stiff much faster than squid so you will have to be very careful with

your dt. The easiest recourse is to employ the *Rosenbrock* (stiff solver); you can engage it via the top pop – up menu in the *Parameters* window. You will also have to use a much stronger stimulus (100 x) to excite. Using a $Q_{10} = 3$, show that, unlike the squid axon cold temperature to say 6°C will block excitation

Appendix

Diffusion of Ions Is a Coupled Process

Diffusion of an electrolyte like KCl is complicated by the fact that its flux is determined by electrical forces as well as the concentration gradients. But, electrical forces are generated by all the ions present. Thus the ion flux also depends on all other ions and, unlike non-electrolytes, ions cannot be treated as independent particles.

First, consider the motion of ions due to electrical forces alone. The electrical force acting on a unit charge is called the electric field and is denoted by *E*. Let *z* represent the valence of an ion (*z* will be positive or negative depending on the sign of the ionic charge), let *F* represent the Faraday constant, and let *N* be Avogadro's number. Then the charge carried by a single ion will be *zF/N,* and its velocity *v***,** will be proportional to the force on it, which is given by *(zF/N)E*, i.e.

Equation 38 $$v = u' \bullet (zF/N) \bullet E$$

where *u*' is a constant.

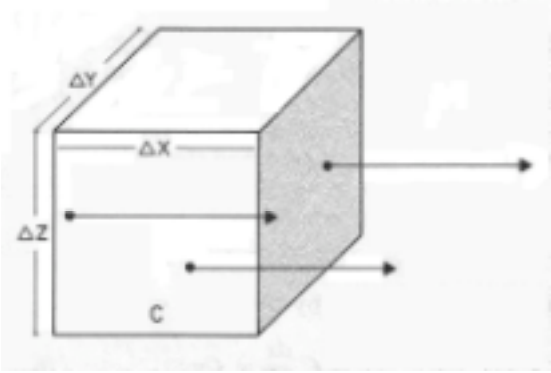

Figure 26. Flux of solute out of an elementary volume. Motion is constrained to the x direction. If the solute velocity is given by Δx/Δt, then all the solutes contained within the volume at time t will have passed through the shaded plane in time t + Δt.

Let *dx* be the distance traveled by an ion in time *dt* so that *v* = *dx/dt*. Now construct an elementary volume, as shown in Figure 26, with dimensions *dx, dy, and dz* and let C denote the concentration of an ion within the volume. If motion is constrained to the x direction, then in time *dt* all the ions contained within the volume (given by *C dx dy dz,)* will have crossed the shaded plane whose area is *dydz*. In time *dt,* the molar flux ,*J*, per unit area through the plane equals

Equation 39 $$J \bullet dy \bullet dz \bullet dt = C \bullet dx \bullet dy \bullet dz$$

Dividing both sides by *dy dz dt,* we obtain

Equation 40 $$J = C\frac{dx}{dt} = Cv$$

Substituting Equation 38 into Equation 40, and noting that E is related to the electrical potential V by the relation $E = -dV/dx$, we have

Equation 41
$$J = \frac{-u' zFC}{N}\frac{dV}{dx} = -uzFC\frac{dV}{dx}$$

where the constant N has been absorbed into u (i.e., $U = u'/N$); the new constant u is called the mobility.

If the ions are subject to concentration gradients as well as electrical potential gradients, their motion is described by a combination of Fick's law and Equation 41:

Equation 42
$$J = -D\frac{dC}{dx} - uzFC\frac{dV}{dx}$$

Both thermodynamic and statistical mechanical arguments show that $D = uRT$. Therefore, noting that $dC/dx = C \cdot dlnC/dx$, Equation 42 can be rewritten in the following equivalent forms:

Equation 43
$$J = -D\left(\frac{dC}{dx} + \frac{zF}{RT}C\frac{dV}{dx}\right)$$

Equation 44
$$J = -D \bullet C\left(\frac{d\ln C}{dx} + \frac{zF}{RT}\frac{dV}{dx}\right)$$

Equation 43, or the equivalent expression Equation 44, is known as the Nernst-Planck equation.

Equilibrium: The Nernst Potential

The simplest application of the Nernst-Planck equation is to ionic equilibria. If an ion is in equilibrium, $J = 0$ and Equation 44 becomes

Equation 45
$$\frac{dV}{dx} = -\frac{RT}{zF}\frac{d\ln C}{dx}$$

Multiplying by dz and integrating both sides through the membrane from outside the cell where $C = C_o$, $V = V_o$ to inside where $C = C_{in}$, $V = V_{in}$, yields

Equation 46
$$V_{EQ} = V_{in} - V_o = -\frac{RT}{zF}\ln\frac{C_{in}}{C_o}$$

The equilibrium potential, computed in Equation 46, is also known as the Nernst potential

Ionic Flux and Current

Consider the membrane illustrated in Figure 27 that extends from the inside surface where $x = 0$ to the external surface where $x = d$. Our task is to integrate Equation 43 through the membrane, solve the resulting expression for J and then relate the concentration and potential at the membrane

boundaries to corresponding quantities within the bathing solutions.

Let V' and C' denote the voltage and concentration within the membrane and let us assume a constant electrical field, i.e. $-dV'/dx$ = constant = $-(V'_o - V'_{in})/d$. This implies that V' will be a linear function of x as illustrated.

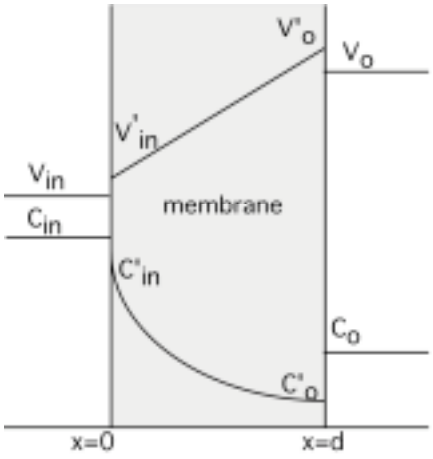

Figure 27 Schematic concentration and voltage profiles through the membrane. The voltage is linear (constant field), while the concentration profile is distorted from linearity by the electric field. The 'in' solution is negative, C represents a cation concentration. The membrane potential, V_m, is given by $V_{in} - V_o$.

Substituting $(V'_o - V'_{in})/d$ for dV/dx in Equation 42, and solving the resultant expression for dC'/dx, we have

Equation 47

$$\frac{dC'}{dx} = -\left(\frac{J}{D}\right) - \left(\frac{zF}{RT}\frac{\left(V'_o - V'_{in}\right)}{d}\right)C'$$

Assuming J is constant within the membrane, then all bracketed quantities are constant (independent of x) and Equation 47 can be integrated to

Equation 48

$$C = C'_{in}e^{\frac{-zF\left(V'_o - V'_{in}\right)}{RTd}x} - J\frac{RTd}{zF\left(V'_o - V'_{in}\right)D}\left(1 - e^{\frac{-zF\left(V'_o - V'_{in}\right)}{RTd}x}\right)$$

where C'_{in} is the concentration just inside the membrane at $x = 0$. The concentration just inside the external surface of the membrane C'_O occurs at $x = d$. Letting $x = d$ in Equation 48, and solving for J yields

Equation 49

$$J = \frac{zF\left(V'_o - V'_{in}\right)D}{RTd\left(1 - e^{\frac{-zF}{RT}\left(V'_o - V'_{in}\right)}\right)}\left(C^{\bullet}_o - C^{\bullet}_{in}e^{\frac{-zF}{RT}\left(V'_o - V'_{in}\right)}\right)$$

The concentrations just inside the membrane are related to corresponding concentrations in the bathing media by a partition coefficient, β as

Equation 50 $$C_{in} = \beta C'_{in} \qquad C_o = \beta C'_o$$

For simplicity, we assume $V'_o - V'_{in} = V_o - V_{in}$, (i.e. any interfacial potential that has to be added is the same on both sides) so that

Equation 51 $$\left(V'_o - V'_{in}\right) = \left(V_o - V_{in}\right) \equiv -V_m$$

Where V_m is the membrane potential. Finally, we define a permeability coefficient P_c by

Equation 52 $$P_c = \beta \frac{D}{d}$$

Substituting Equation 50,Equation 51, and Equation 52 into Equation 49 yields the GHK flux equation:

Equation 53 $$J = P\frac{zF}{RT}V_m \frac{C_o - C_{in}e^{\frac{zF}{RT}V_m}}{1 - e^{\frac{zF}{RT}V_m}} = P\frac{zF}{RT}V_m \frac{C_{in}e^{\frac{zF}{RT}V_m} - C_o}{e^{\frac{zF}{RT}V_m} - 1}$$

Using the relation $I = zFJ$, this equation can be easily recast to compute the current , I_C, carried by the ion. In this case it is convenient to multiply and divide the numerator by C_o and to use Equation 46 to substitute $\exp(-zFV_{eq}/RT)$ for C_o/C_{in} , i.e.

Equation 54 $$I_c = zFC_oP\frac{zF}{RT}V_m \frac{\frac{C_{in}}{C_o}e^{\frac{zF}{RT}V_m} - 1}{e^{\frac{zF}{RT}V_m} - 1} = g_cV_m \frac{e^{\frac{zF}{RT}(V_m - V_{EQ})} - 1}{e^{\frac{zF}{RT}V_m} - 1}$$

where $_c$

Equation 55 $$g_c = \frac{z^2F^2}{RT}PC_o$$

BEGIN BOX

J[μmol·cm^{-2}·sec^{-1}], *P*[cm·sec^{-1}], V_m[mV], *C*[μmol·cm^{-3}] and *time*[sec] form a set of practical units□

First consider voltage units and note that V_m [mV] is consistently multiplied by zF/RT[mV^{-1}] making the product zFV_m/RT dimensionless. To see this, recall that:

z is a pure number

$F = 96485[\text{Coul}\cdot\text{mol}^{-1}]$

$R = 8315\ \text{mV}\cdot\text{Coul}^{-1}\cdot\text{mol}^{-1}\cdot\ ^{\circ}\text{K}^{-1}$.

Thus

$zF/RT = z \cdot 96485[\text{Coul}\cdot\text{mol}^{-1}]\ /8315[\ \text{mV}\cdot\text{Coul}^{-1}\cdot\text{mol}^{-1}\cdot\ ^{\circ}\text{K}^{-1}]\cdot T[^{\circ}\text{K}] = z\cdot 11.60\cdot\ [\text{K}^{-1}]\cdot T[^{\circ}\text{K}]\cdot[\text{mV}^{-1}]$

For example, for a univalent cation, say at 20ºC = 293ºK, we have $zF/RT = 0.04\text{mV}^{-1} = 1/25\text{mV}$.

With dimensionless zFV_m/RT, practical units for Equation 53 are straight forward. Common units for P are $[\text{cm}\cdot\text{sec}^{-1}]$ and if we choose $[\mu\text{mols}\cdot\text{cm}^{-2}\cdot\text{sec}^{-1}]$ for flux, then consistency is obtained with concentration units in $[\mu\text{mols}\cdot\text{cm}^{-3}]$ and *time* units in [sec]. i.e.

Equation 56 $J[\mu\text{mols}\cdot\text{cm}^{-2}\cdot\text{sec}^{-1}] = P\ [\text{cm}\cdot\text{sec}^{-1}]\cdot Conc[\mu\text{mols}\cdot\text{cm}^{-3}] = P\ [\text{cm}\cdot\text{sec}^{-1}]\cdot Conc[\text{mM}]$

This choice of units is advantageous because it consistently uses cm as the linear measure, while the concentration unit µmols·cm-3 is numerically equivalent to the more commonly used millimols·L^{-1} = mM.

I[µamp·cm^{-2}], *P*[cm·sec^{-1}], V_m[mV], *C*[µmol·cm^{-3}] and *time*[msec] form a set of practical units for electrophysiology

To define the units for Equation 54, recall that if we specify μfarads and mV for the units of membrane capacitance , C_m, and voltage , V_m, then the relation $Q = C_m \cdot V_m$, requires that Q be in units of nCoul. Further, since msec are the most convenient time units for many electrophysiology models, our dimensional representation of Equation 54 becomes:

Equation 57 $I[\text{nCoul}\cdot\ \text{cm}^{-2}\cdot\text{msec}^{-1}] = F\cdot[\text{nCoul}\cdot\text{nmol}^{-1}]\cdot\ P[\text{cm}\cdot\text{msec}^{-1}]\cdot\ C[\text{nmol}\cdot\text{cm}^{-3}]$

where zFV_m/RT again, has been left out because it is dimensionless.

Equation 57 clearly sets msec as the time base of the model. However we can substitute other equivalent , more commonly used, quantities without compromising this time base. Membrane currents are generally measured in

$[\mu\text{amp}\cdot\text{cm}^{-2}] = [\text{nCoul}\cdot\ \text{cm}^{-2}\cdot\text{msec}^{-1}\ ]$.

Concentrations are measured in $[\text{mM}] = [\mu\text{mol}\cdot\text{cm}^{-3}]$ while permeability is usually in $[\text{cm}\cdot\text{sec}^{-1}]$. But,

$[\text{cm}\cdot\text{msec}^{-1}] = 10^{3}\ [\text{cm}\cdot\text{sec}^{-1}]$,

$[\text{nmol}\cdot\text{cm}^{-3}] = 10^{-3}\ [\mu\text{mol}\cdot\text{cm}^{-3}]$

Substituting these values into Equation 57, we arrive at the alternative

Equation 58 $I[\mu amp \cdot cm^{-2}] = F \cdot P[cm \cdot sec^{-1}] \cdot C[\mu mol \cdot cm^{-3}] = F \cdot P[cm \cdot sec^{-1}] \cdot C[mM]$

Simply comparing Equation 57 and Equation 58, it is not clear whether the time base is in sec or msec. However, as we have seen the choice of units for V_m and C_m require Q to be in nCoul. The program updates the voltage on the assumption that current input is $nCoul \cdot msec^{-1}$.

END BOX

Electrophysiological definitions and units

DEFINITION	Symbol	Value
Avogadro's Number	*N*	6.02×10^{23} mol^{-1}
Faraday's constant	*F*	9.65×10^{4} Coul/mol
elementary charge	*e*	1.602×10^{-19} Coul
gas constant	*R*	8.315 J/(mol·°K)
joule	*J*	1 V/Coul
volt	*V*	1 J/Coul
ampere	*A*	1 Coul/sec
At 20° C	*F/RT*	0.040 mV
At 37° C	*F/RT*	0.045 mV

Table 5. Some electrophysiological constants.

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