

## BMEN E4001x: Quantitative Physiology I / Molecular and Cellular Systems

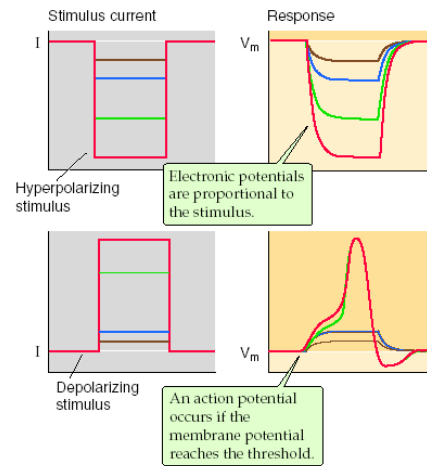
### Notes 10 - Excitable membranes

#### B&B Chapter 7

Consider two manipulations:

**hyperpolarization:** current is pulled (holes are pulled, electrons are injected) to make the potential more negative than resting. The result is typically a **graded response**, in which the membrane adjusts to accommodate the new electrical state. Higher injection rate, higher deviation from the non-current resting potential.

**depolarization:** current is pushed (holes injected, electrons pulled) to raise the intracellular voltage. In some cells, this is met with a graded response. In electrically excitable cells, this is met with a stereotypical excursion, the **action potential**



There are many, many, many manipulations that can happen using the patch clamp technique, but we'll first consider the case in which a current is injected to initiate the action potential, then voltage is watched to follow the cell membrane behavior. The stereotypic voltage, read at a single position of the membrane, is shown below:

It consists of multiple phases, including:

**Depolarization and Threshold:** when the membrane voltage crosses this level, the voltage now takes off precipitously towards higher voltages.

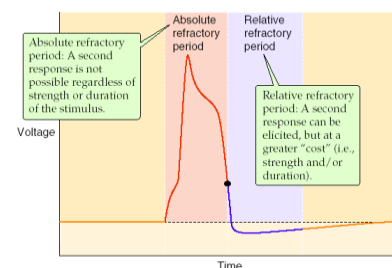
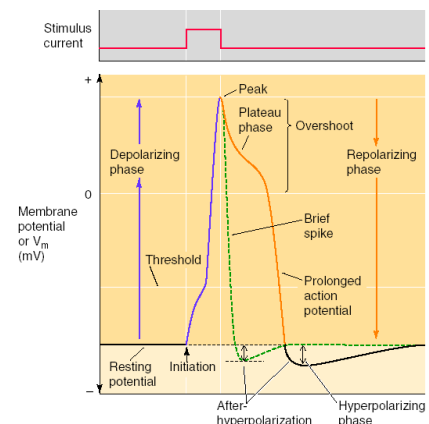
**Peak:** at some point, the voltage peaks, then begins to become more negative. The peak voltage is often greater than zero.

**Repolarization:** Voltage drives back towards resting, with several subphases. The final potential is often lower than the initial resting potential; this is called hyperpolarization.

The duration of this entire sequence can range from a few milliseconds to something on the order of a second, depending on the specific cell type, species, pathology. The specific levels that these voltages correspond to can also vary with any of a huge range of variables.

This feature travels at 5-25 m/s. At any given point, an AP in squid axon is only a few milliseconds. Thus, the entire feature has an extent on the order of millimeters to centimeters.

In the absolute refractory period, another action potential simply cannot be initiated. In the relative refractory period, it is possible to initiate a new action potential, but requires more current/duration.



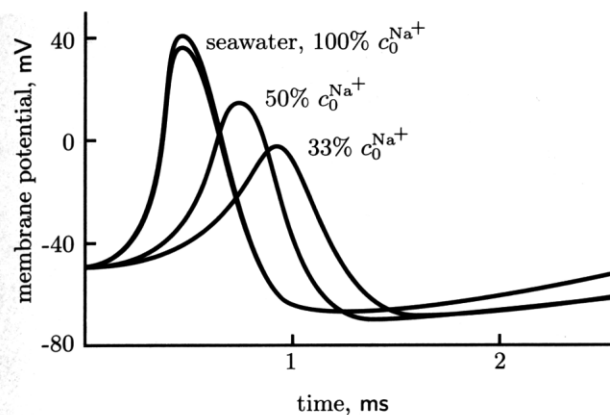
Our goal here is to explore some of the molecular aspects of this

relative to our discussion on membranes, specific implementations in specific physiological systems, including the propagating nature of this in neurons, is the domain of QPIL.

Much of the pioneering work was done in the giant squid axon, due to its size and accessibility. The ion composition in this system is a bit different than the mammalian cell, but Nernst potentials are quite close:

Ion	concentration (mM)		
	interstitial space	cell ("typical")	$V^{\text{nernst}}$ (mV)
$\text{Na}^+$ , mammalian cell	145	15	+59 (37C)
$\text{K}^+$ , mammalian cell	4.5	120	-71 (37C)
$\text{Na}^+$ , squid giant axon	440	50	+54 (15C)
$\text{K}^+$ , squid giant axon	20	400	-75 (15C)

- As we have been discussing, the resting potential suggests that the cell is not at equilibrium, but close to the Nernst potential for  $\text{K}^+$ . This source of the resting potential was proposed by Bernstein, **1902**, followed by the idea that the membrane becomes permeable to all ions during an action potential.
- late 1930's, Cole & Curtis, and demonstrated that action potentials are associated with an increased conduction.
- Later, Cole & Curtis, and simultaneously by Hodgkin & Huxley, measured the transmembrane voltage, and found that it actually crossed zero, going to near +40mV, near that for Sodium. This sets that idea that sodium permeability may be important
- 1940s, Hodgkin & Katz show that altering the external  $\text{Na}^+$  concentration (bringing it down from seawater, but maintaining electroneutrality), altering action potential shape (see graph).

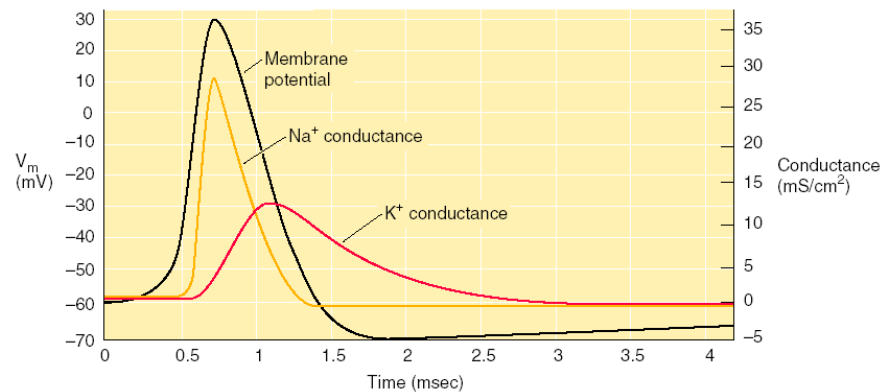


Additional pharmacological approaches elucidate properties of the sodium and potassium channels. Note that current has a total current and ionic current. The initial peak corresponds to capacitor charge/discharge, while the remainder of the curve, in the patch clamp experiment, corresponds to ionic currents.

Note that these experiments show:

- Sodium conductance is dependent on voltage, with a sharp increase at  $-30$ , but looking like ohmic conduction later. Also, more conductance with higher voltage.
- Sodium conductance is time limited.
- Potassium conductance appears voltage dependent and slow, but doesn't automatically close.

What was codified by Hodgkin and Huxley (1952) in the squid axon follows:



H&H developed a series of time- and voltage-dependent expressions for  $\text{Na}^+$  and  $\text{K}^+$  conductance. Both K&S and Nelson have good coverage of these equations, but we will stick to a non-quantitative system here.

Upon reaching some membrane threshold,  $\text{Na}^+$  conductance increases sharply, and ions flow in. At peak, the conductance of  $\text{Na}^+$  increases from  $\sim 1/20 g_{\text{K}_a}$  to  $20 \times g_{\text{K}_a}$ . This is a positive feedback loop on  $\text{Na}^+$  conductance, leading to a fast rise.

On a longer timescale, potassium conductance increases, and ions flow out. The peak is at about one millisecond, and extends out to about 2.5 milliseconds.

This potassium flux is what drives the membrane potential back, reaching the K Nernst potential. The  $\text{Na}^+$  channel is self-limiting in that it closes with time, while the  $\text{K}^+$  channel is not. The  $\text{K}^+$  channel stay open as long as the voltage is above a certain point.

While the  $\text{Na}^+$  channel is closed, we're in the absolute refractory period.

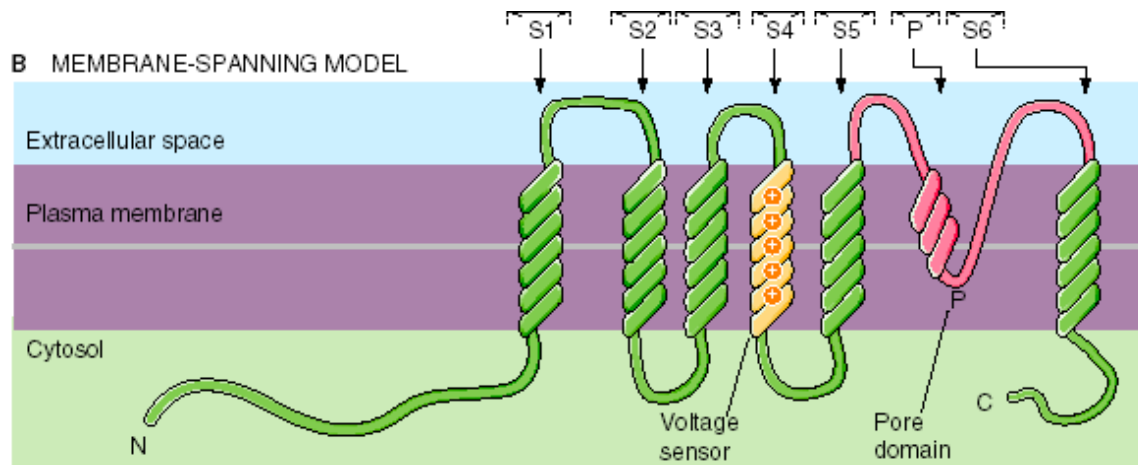
While the  $\text{Na}^+$  channel is openable again, but the  $\text{K}^+$  channel is still closing,

This is the basis of excitable membranes. Remember that this waveform must be a traveling system, but we won't go into conduction here, this is a topic of QPIL.

## Voltage-gated channels

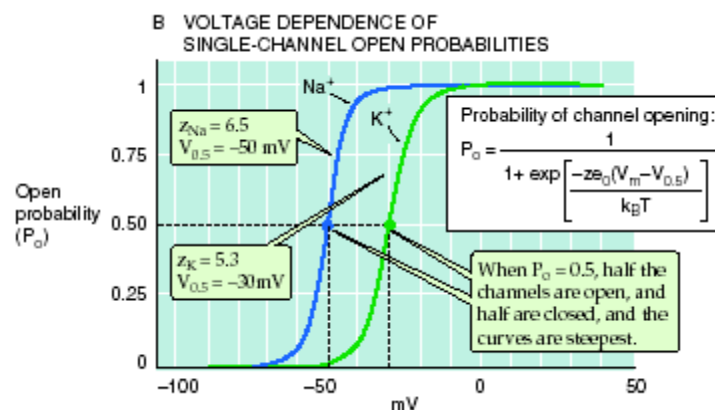
B&B has a good discussion on the various families of channels that give rise to this behavior.

Some mechanisms:



K<sup>+</sup> channels in the Shaker fruit fly, 1987. Pink region (called P region, no relation) dictates specificity. The charged helices are believed to be the voltage sensor, and can move up and down in the plane of the membrane.

In addition, Na<sup>+</sup> channels have an inactivating mechanism. See B&B, chapter 7, for more details, in addition, some information on getting the crystal structure of these.

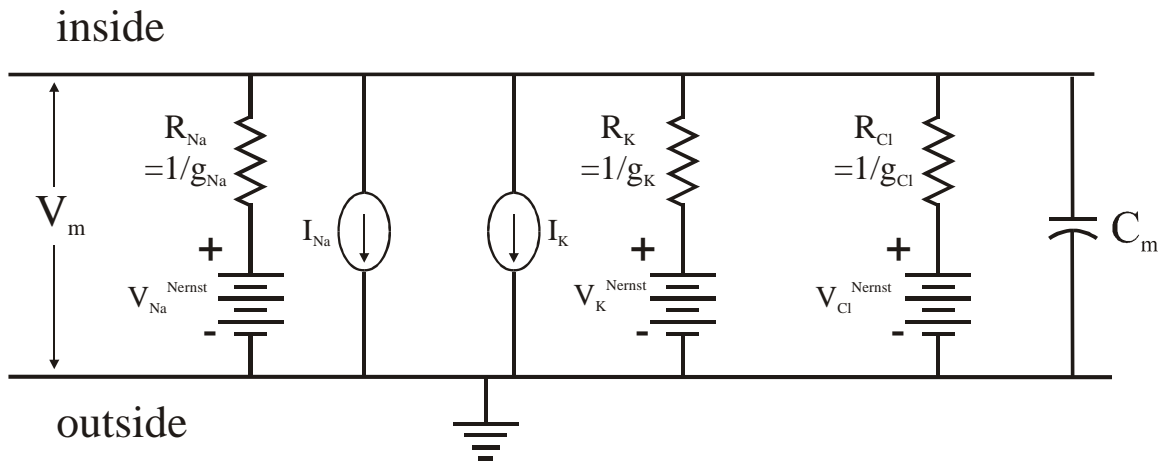


## Traveling action potentials and more beyond this course:

One thing we're not covering here is how potentials get propagated. This will be covered in detail more in QP11, and falls to the realm of cable theory. B&B does a good job with linear cable theory, enough for most of our purposes. P. Nelson (Chapter 12) goes further with a nice discussion on non-linear cable theory, giving rise to a traveling action potential. K&S goes even further, with a good coverage of the Hodgkin-Huxley equations in section 4.1, as well as extensions of this system.

## How fast does membrane voltage change? Electrical circuit of the cell membrane.

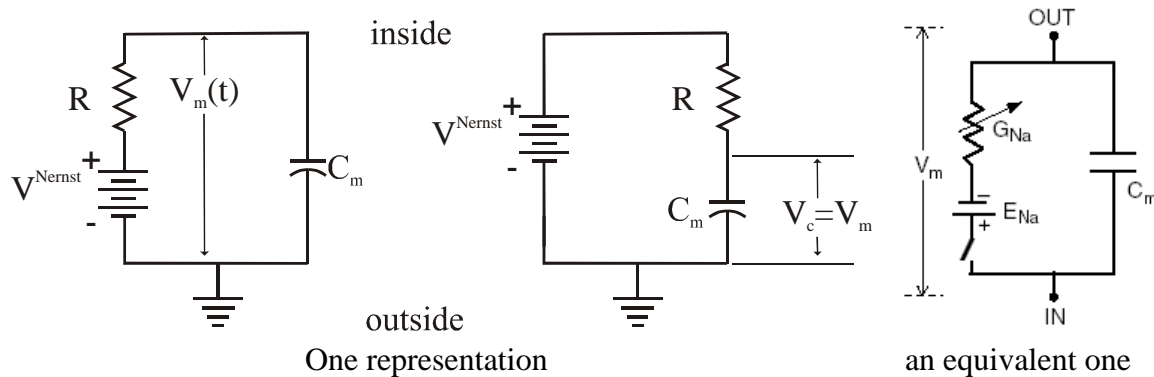
Effectively, the membrane can be viewed as a capacitor; charges separated by a dielectric material (not water, but an oil). It will be included in our larger schematic of the membrane in the following manner:



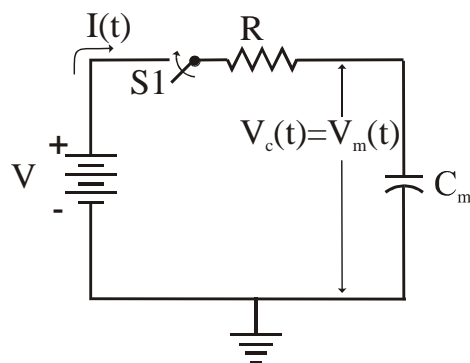
In our upcoming discussions, we're going to look at how this system changes as conductances change. The capacitance is a big player in this.

First start with a simplified system:

## The circuit:



Let's solve for a more general case:



We'll generalize this for any ion, assuming:

- at time 0, membrane voltage is  $V_0$
- at time 0, the switch is closed, the potential "V" is applied
- How does this system respond?

$$I(t) = g[V - V_m(t)] \text{; resistor}$$

$$I(t) = C_m \frac{dV_m(t)}{dt} \text{; capacitor}$$

Rearranging,

$$\frac{dV_m(t)}{dt} = \frac{g}{C_m} [V - V_m(t)]$$

And now, using  $V'(t) = V_m(t) - V$ ,

$$\frac{dV'(t)}{dt} = -\frac{g}{C_m} V'(t)$$

With a solution

$$V'(t) = A \exp\left(-\frac{g}{C_m} t\right)$$

Which, using  $V_m(0) = V_0$ , gets us the equation

$$V_m(t) = (V_0 - V) * \exp\left(-\frac{g}{C_m} t\right) + V$$

$$\text{Time constant} = \tau = C_m/g$$

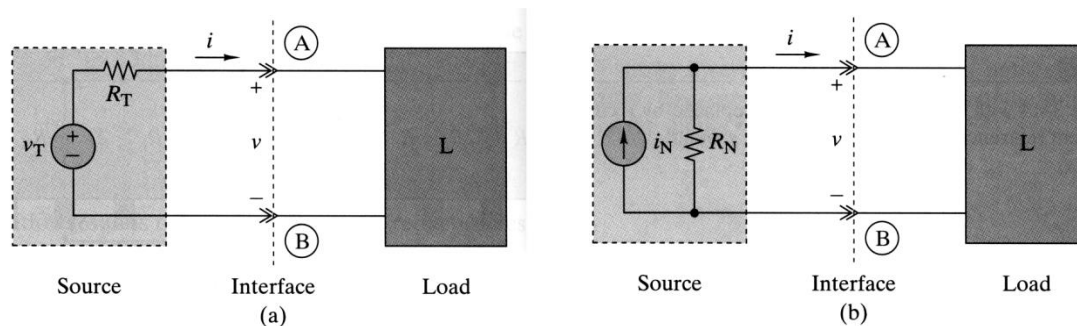
And more generally, this same exponential character will be found for any change. For a general analysis:

Remember that a capacitor doesn't like quick changes in voltage. So, if conductance/resistance or voltage changes quickly, the capacitor voltage will start at the same voltage it was before the change, then approach another. So:

1. Determine the membrane voltage before the change
2. Consider the capacitor removed from the circuit, that will give you the final voltage (look to pump-leak model, GHK, etc.)
3. The capacitor/membrane voltage goes from the initial to final with an exponential equation, typically of the form  $\Delta V * (1 - \exp(-t/\tau))$ , where  $\tau = C_m/g = RC_m$ . Note that these have to be adjusted for area.

The trick is, then, what is R? For a single source and resistance, this is a no-brainer. Also, if there is a conductance that completely dominates the system, the entire system can be approximated as consisting of only this Nernst potential and conductance.

We can get at this from linear circuit theory, using Thevenin and Norton equivalent circuits, in which we can convert between current and voltage sources.



From these treatments,  $R_N = R_T$

Now, use these relations to simplify the original circuit.

Each Nernst potential/resistor converts to a current source in parallel with a resistor:

$$V_i^{\text{Nernst}}, R_i \Rightarrow I_i = V_i^{\text{Nernst}} / R_i = g_i V_i^{\text{Nernst}}, R_i$$

Add them together in parallel, add pumps.

$$I_C = \sum_i I_i - \sum_k i_k^{\text{pump}} = \sum_i g_i V_i^{\text{Nernst}}; R_c = \frac{1}{\sum_j 1/R_j} = \frac{1}{\sum_j g_j}$$

Most important point is that this gives an equivalent resistance. Getting to a source voltage, which should be the non-capacitor system you computed earlier, is a bit tricky, given the constraints on what the pump currents can be.

### Capacitance of parallel plate system:

$$C = \epsilon_0 \epsilon A / d, \text{ or } c = \epsilon_0 \epsilon / d$$

$\epsilon_0$  = permittivity constant of space

$$\epsilon_0 = 8.854 \times 10^{-12} \text{ C}^2 / (\text{N} \cdot \text{m}^2)$$

$$\epsilon_{\text{lipid}} = 5$$

$$d \sim 5 \text{ nm}$$

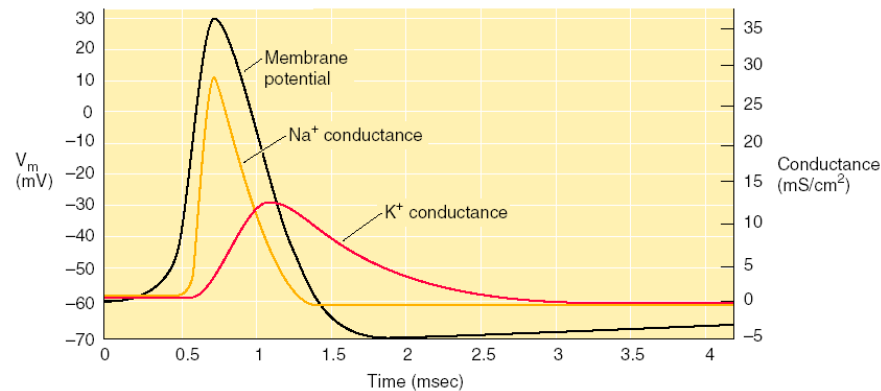
$$C = 8.85 \times 10^{-2} \text{ F/m}^2 \sim 1 \mu\text{F/cm}^2$$

So,  $\tau_{\text{Na}} = (1 \mu\text{F/cm}^2) / (25 \text{ mS/cm}^2) = 40 \mu\text{s}$ . Consider that the rise time is on the order of 500  $\mu\text{s}$ , and this is the peak sodium flux, which is occurring in the face of potassium flux, not too bad an estimate!

### With the numbers:

Note: we're going to reveal some of the problems and nuances of applying the treatments we just described. There is a bit of art and science in applying these, and we won't go too far into it. The goals are to have some insight into when each model is appropriate, realize that they give sometimes different answers, and rationalize where these differences come from. For grading and question purposes, this course will be specific on which system to use.

A rough approximation of the curves arrived at with the Hodgkin & Huxley model, following the giant squid axon. In short, recreate aspects of the time scale of:



Assume resting potential of  $-70$  mV, peak of  $+40$  mV, hyperpolarization to  $-75$  mV  
Use the following conditions for ions in solution:

Ion	concentration (mM)		$V^{\text{Nernst}}$ (mV)
	outside	inside	
$\text{Na}^+$	440	50	+54
$\text{K}^+$	20	400	-75
$\text{Cl}^-$	560	52	-59

All calculatable from the equation for the Nernst potential:

$$V^{\text{Nernst}} \equiv -\frac{k_B T}{zq} \ln \left( \frac{C_{\text{internal}}}{C_{\text{external}}} \right); k_B = 1.3807 \text{E-}23 \text{ J/K}, q = 1.602 \text{E-}19 \text{ C}; \text{ use room temp. of } 15\text{C}$$

$$\Rightarrow k_B T/q = 24.8 \text{ mV @ } 15\text{C (288K)}, 25.9 \text{ mV @ } 27\text{C (300K)}, 26.7 \text{ mV @ } 37\text{C (310K)}$$

## Conductances:

There are a wide range of reported values. Here, we'll use these:

- $\text{Na}^+$  conductance, peak, action potential, squid axon:  $27 \text{ mS/cm}^2$
- $\text{K}^+$  conductance, peak, action potential, squid axon:  $12 \text{ mS/cm}^2$
- $\text{Na}^+$  conductance, resting, squid axon:  $0.1 \text{ mS/cm}^2 = 100 \mu\text{S/cm}^2$
- $\text{K}^+$  conductance, resting, squid axon:  $1.5 \text{ mS/cm}^2$
- $\text{Cl}^-$  conductance, squid axon:  $.8 \text{ mS/cm}^2$

## Start with resting cell

From pump-leak model, assuming Na/K pump with 3/2 ratio:

$$V_R = \frac{3V_{\text{K}^+}^{\text{Nernst}} g_{\text{K}^+} + 2V_{\text{Na}^+}^{\text{Nernst}} g_{\text{Na}^+}}{(2g_{\text{Na}^+} + 3g_{\text{K}^+})}$$

With the values given,  $\Delta V = -70$  mV, pretty good.



Note that the  $\text{Cl}^-$  concentrations are a bit off, as the measured or stated ones are not in keeping with the resting potential.

### ***Now, open Na channels.***

Use the peak Na conductance as an estimate to get the rising edge:

- $g_{\text{Na,AP}} = 27 \text{ mS/cm}^2$

Consider a  $500 \mu\text{m}$  diameter axon, take a  $1 \mu\text{m}$  length

$$\text{Area} = \pi * 500 \mu\text{m} * 1 \mu\text{m} = 1570 \mu\text{m}^2$$

$$\text{Capacitance} = 1 \mu\text{F/cm}^2 * 1570 \mu\text{m}^2 * (1\text{E-}8 \text{ cm}^2/\mu\text{m}^2) = 1.6\text{E-}11 \text{ Farad}$$

$$\text{Resistance for this patch} = 1/(g_{\text{Na,AP}} * 1570 \mu\text{m}^2) = 1/(4.2\text{E-}7 \text{ S}) = 2.36 \text{ M}\Omega$$

Time constant =  $3.7\text{E-}5$  seconds  $\sim 35 \mu\text{s}$ . Certainly fast enough to provide the designated graph.

What is the target potential?

- $g_{\text{Na}} = 27 \text{ mS/cm}^2$
- $g_{\text{K}} = 1.5 \text{ mS/cm}^2$
- $g_{\text{Cl}} = 0.8 \text{ mS/cm}^2$
- Pump-leak  $\Rightarrow 44 \text{ mV}$ , pretty good

Now, during repolarization of membrane

Use the peak K conductance,  $g_{\text{K,AP}} = 12 \text{ mS/cm}^2$

Assume an “average” Na conductance,  $g_{\text{Na,RP}} = 7 \text{ mS/cm}^2$

Time constant, assuming that we can use the electrical model without following each species separately (conductances add up)  $\Rightarrow C = 1.6\text{E-}11 \text{ Farad}$ ,  $R = 3.35 \text{ M}\Omega$ , time constant =  $53 \mu\text{s}$ . Very fast, this stage dominated by channel protein details.

### ***After hyperpolarization:***

Final potential set by  $g_{\text{K}} = 5 \text{ mS/cm}^2$ ,  $g_{\text{Na}} = 0.1 \text{ mS/cm}^2$ . Hyperpolarization voltage set to: pump leak  $\Rightarrow -73 \text{ mV}$

Now, turn off all action potential channels. Time constant of this system, assuming that we can follow the total ions:  $C = 1.6\text{E-}11 \text{ Farad}$ ,  $R = 1/((1.5 + .1 + .8 \text{ mS/cm}^2) * 1570 \mu\text{m}^2 * (1\text{E-}8 \text{ cm}^2/\mu\text{m}^2))$ ,  $\tau = 4\text{E-}4 \text{ sec}$ . Thus, on the order of milliseconds to recover. Reasonable.