STUDIES OF CHANGING MEMBRANE POTENTIAL*:

1. BASIC ELECTRICAL THEORY,
2. GRADED AND ACTION POTENTIALS
3. THE VOLTAGE CLAMP AND MEMBRANE POTENTIALS

I. INTRODUCTION

A. So far we have only considered the transmembrane potentials that all cells exhibit. These potentials tend to be constant and, by themselves, are not very important in terms of communication between cells. We will refer to these potentials as **RESTING POTENTIALS**, defined as the relatively constant transmembrane potential seen in all cells; however, the term is particularly appropriate for excitable cells.

B. It was well known by the beginning of the century that nerve and muscle cells do not exhibit constant potentials. Under certain conditions, the potentials in these cells can rapidly decrease towards zero and then polarize in a (+) direction, often briefly reaching values as high as +60 mV. This is followed by a rapid return to the original resting potential. Depending on the type of excitable cell, this series of events could be over in as few as 2 milliseconds to as long as many seconds. Furthermore, these potentials move down the excitable cell with time. Thus, one way to think about them is to think of an electrical disturbance that passes along the cell. We call these disturbances **ACTION POTENTIALS**. For the next couple of classes, we will examine action potentials.

II. BASIC ELECTRICITY NEEDED TO UNDERSTAND EXCITABLE CELLS

A. CURRENTS, ELECTROMOTIVE FORCES AND CIRCUITS

1. **CURRENT: moving charged particles. For our purposes, these moving charged particles will be various ions such as K^+, Na^+, Ca^{++}, and Cl^-.** In electricity, the moving charges are electrons. Current is measured in units of **AMPERES**, which are equal to a certain number of charges (in Columbus) moving past a certain point in 1 second.

2. **ELECTROMOTIVE FORCE (emf): the force that causes ions (or electrons) to move, measured in volts (V).** We will often simply refer to emf as voltage.

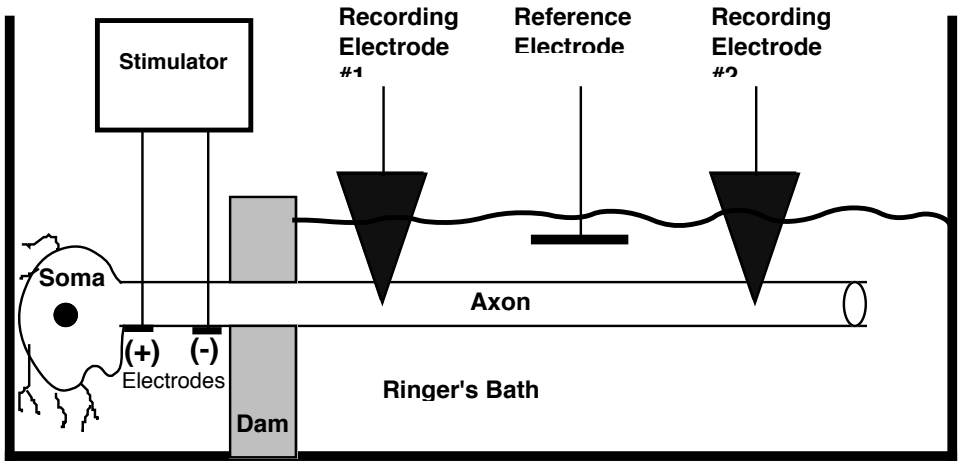
3. **CIRCUIT**: the pathway followed by the current. There are different features that can be incorporated into a circuit that affect the characteristics of the circuit. We will be concerned with two such circuit elements, resistance and capacitance.

B. RESISTANCE AND CAPACITANCE

III. ELECTRICAL STIMULATION OF EXCITABLE CELLS: THE ELECTROTONIC RESPONSE

A. By the beginning of this century, it was well known that the E_m on excitable cells often changed rapidly and in a predictable manner. Studies on E_m were done where two different sets of electrodes were employed, one set to **stimulate** the cell and the other to **record** the membrane potential.

Here is an example of such a set-up. Note the location and order of the stimulating electrodes.



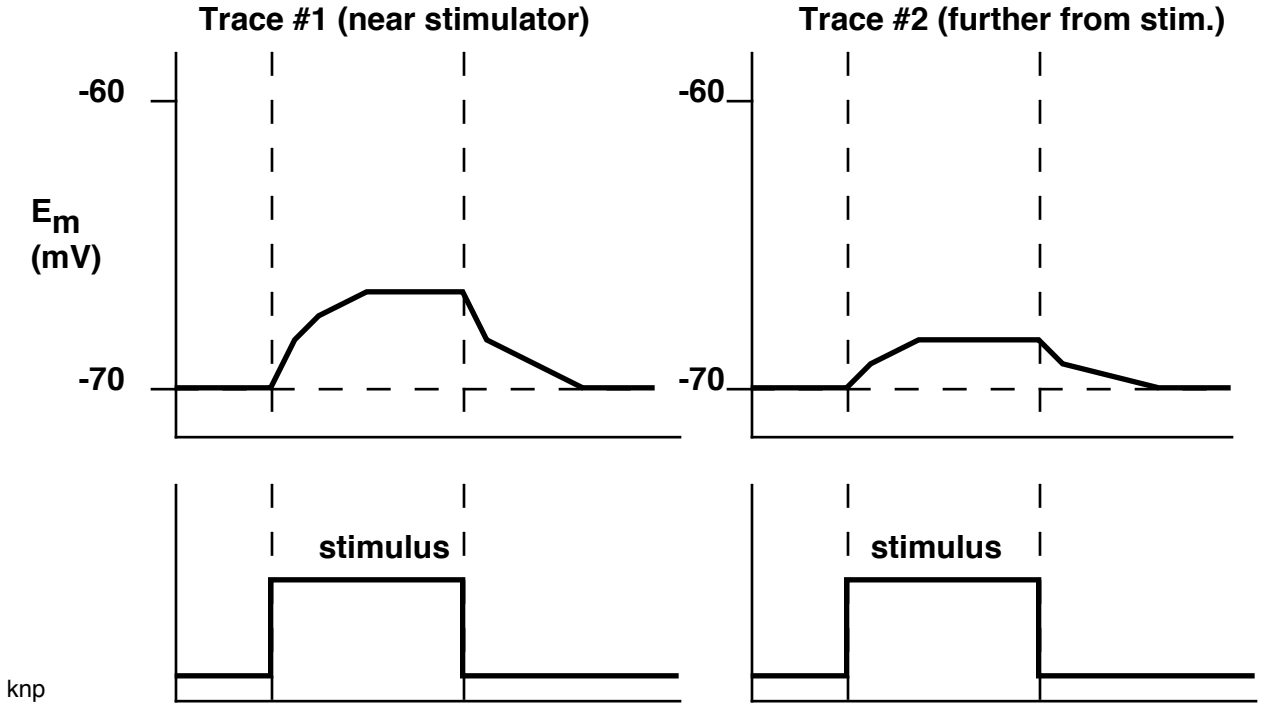
knp

Please imagine the electrodes to be in direct contact with the neuron.

The axon hillock is often used because it is a particularly excitable area.

Both stimulating electrodes are outside of the neuron. They function by drawing away (+) charges from the outside of the membrane, thereby **depolarizing** (moving E_m towards 0) it. The cathode of the stimulating electrodes is always placed between the stimulating anode and the recording electrodes for reasons that will be obvious shortly.

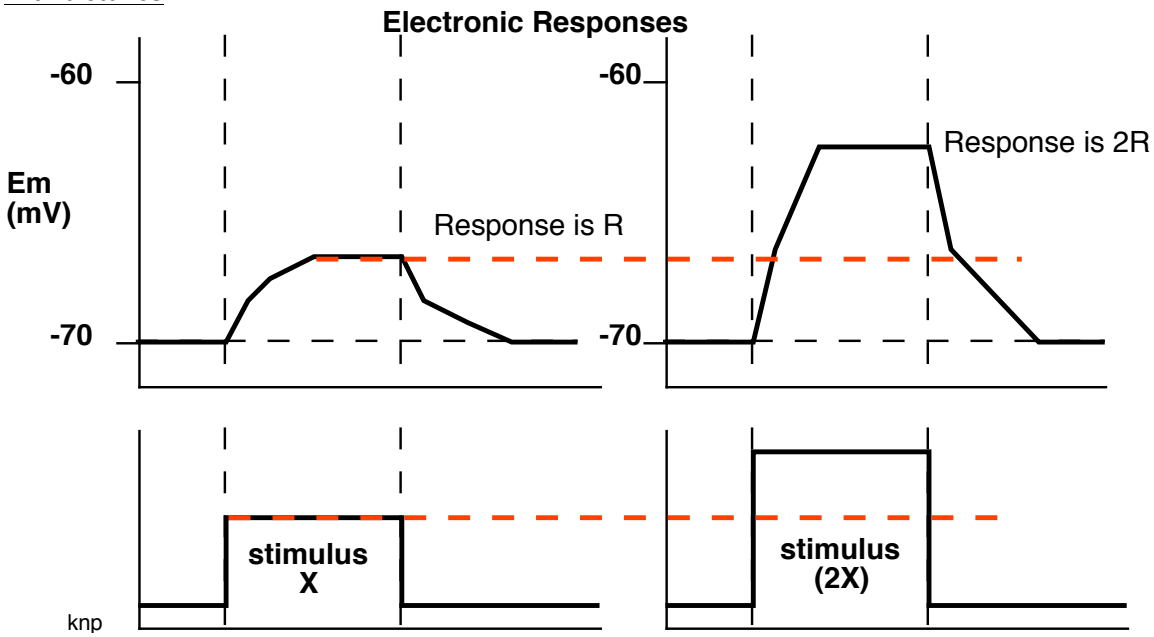
B. Let's try an experiment. Here, we will apply a single, sub-threshold stimulus and see the result at two distances (on the axon) from the stimulus point (the hillock):



knp

! Note that the stimulus shown under the second recording electrode response is not the stimulus measured there -- it is only for comparisons of shape and amplitude. Both stimulus recordings would be made directly from the stimulator.

Notice that the response does not move any appreciable distance down the neuron -- it dies out with distance.



Even more interestingly, notice the shape of the responses are not at all like the shape of the stimulating signal. It is a square wave, but these responses have rounded (logarithmically rounded) on and off portions.

? You have seen curves similar to these recently.
What do they remind you of?
ANS: They are identical to those seen in charging and discharging capacitors. Thus, the axon transmits electricity from a stimulus over sort distances, but it does not do so like a wire would.

? What would it look like if the axon acted like a wire?

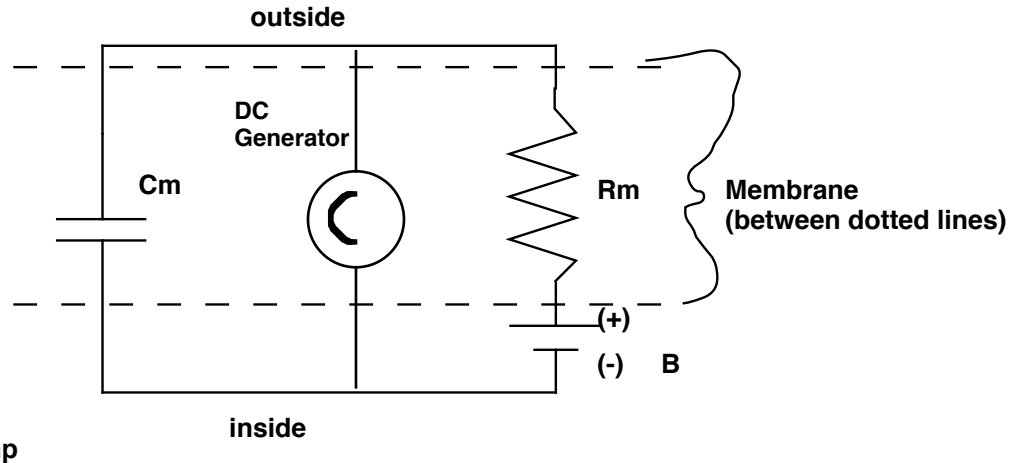
1. This very local response is called an **ELECTROTONIC OR PASSIVE RESPONSE**. Its two most important characteristics are that:

a. Its peak amplitude is essentially equal to the value of the stimulating voltage (ideally, in a good prep.)

b. It is not propagated any meaningful distance; that is, the amplitude decreases with distance.

The electrotonic response will be dealt with later when we talk about **cable properties** of excitable cells.

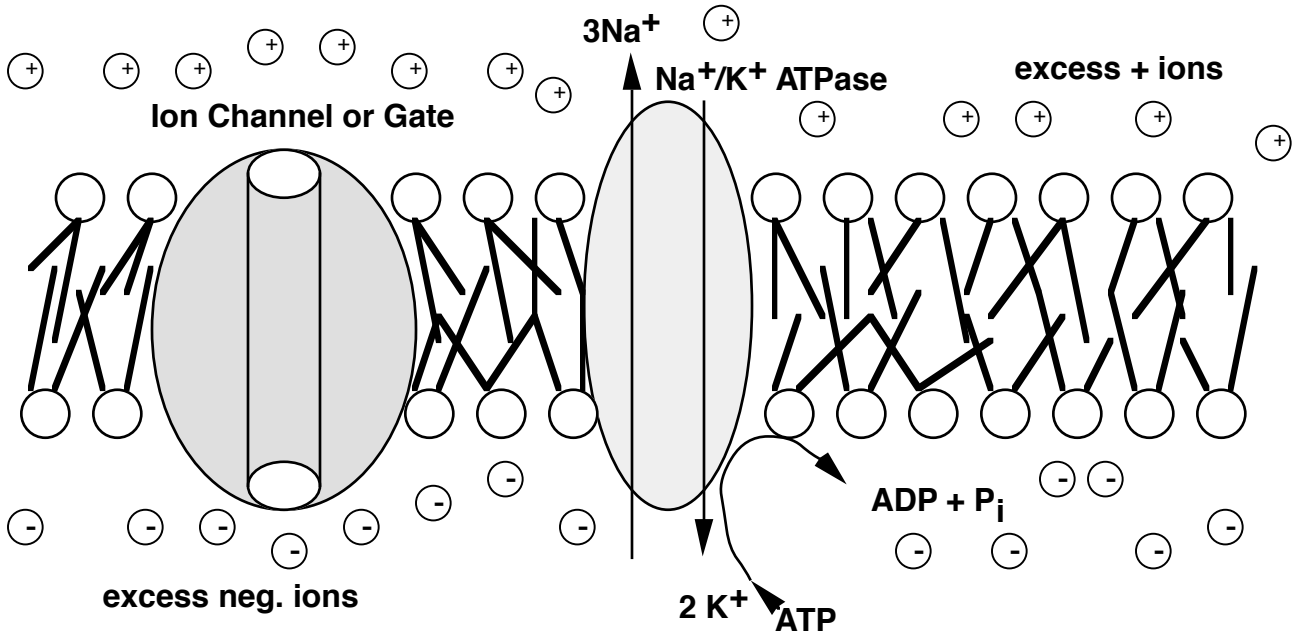
C. We can now draw an **equivalent circuit of the axon** based on what we have seen and already know about its structure. For our drawing we will use resistors, capacitors, a battery and wire. The circuit diagram must reflect what we have observed in terms of the electrical behavior of the axon and the components must correspond to things found in or around the cell. Here's the first and simplest version:



knp

1. In this version, the DC generator represents the ions pumps that produce the non-equilibrial distributions of ions, the battery represents those ionic distributions, the resistor represents the places where electrical flow can occur from the inside to the outside of the membrane (i.e. a protein channel for ion flow), and the capacitor is the lipid bilayer (the dielectric) and the intra and extracellular fluid (the conductor plates of the capacitor).

2. Let's see how it all works. Normally at rest there is a potential across the membrane that has been placed there by the pumps (mainly the Na^+ / K^+ ATPase) that have charged the ionic "batteries". Charges are stored on opposite sides of the membrane, with (-) charges predominating on the inside. It is possible for ions to move in or out of the cell through a resistance (**What are the resistances?**) -- but none do because the cell is at equilibrium.



knp

3. Now some of the (+) charges are drawn away from the outside by the (-) electrode. What happens to the voltage across the capacitor (membrane)?

ANS: since there are fewer (+) charges there now, there is less charge separation and the voltage drops towards zero -- the amount of the drop ultimately being equal to the size of the stimulus.

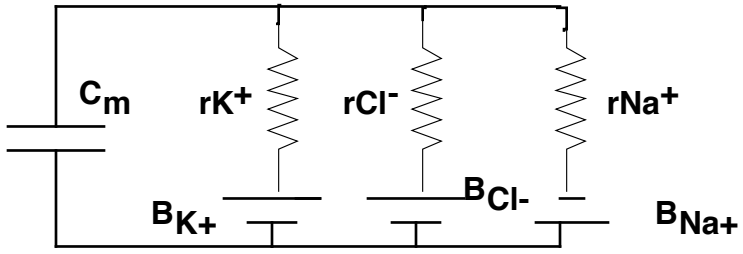
? Why doesn't the drop occur instantaneously?

If E_m was -60 mV and the stimulus was 5 mV, what will be the new E_m equilibrium voltage below the stimulating cathode?

What is it below the stimulating anode? (Remember, the recording electrode is always inside the cell.)

Try to draw this situation for yourself.

4. Before we temporarily drop this model, lets make two modifications that will make it more realistic. The have to do with adding extra resistances and batteries. These additional components will be used to indicate that we know that more than one ion is involved in membrane potentials:



knp

Realize that the values for each of these batteries and resistors are all quite different from each other. **The particular value of a certain resistor represents the permeability of the ion channel that the particular resistor represents. The value of the battery is determined by the equilibrium potential for the particular ion.**

? Given what you know about permeabilities at rest and about equilibrium potentials for the different ions, would you expect the resting "resistance" of the Na^+ resistor to be high (impermeable) or low?

What is the value of this battery? (hint -- what equation could you use to predict it?).

Why do the different batteries have different polarities with respect to the membrane? (Notice that K^+ and Cl^- have the (-) pole pointing inward while Na^+ has the opposite configuration.)

IV. ACTIVE AXONAL RESPONSES

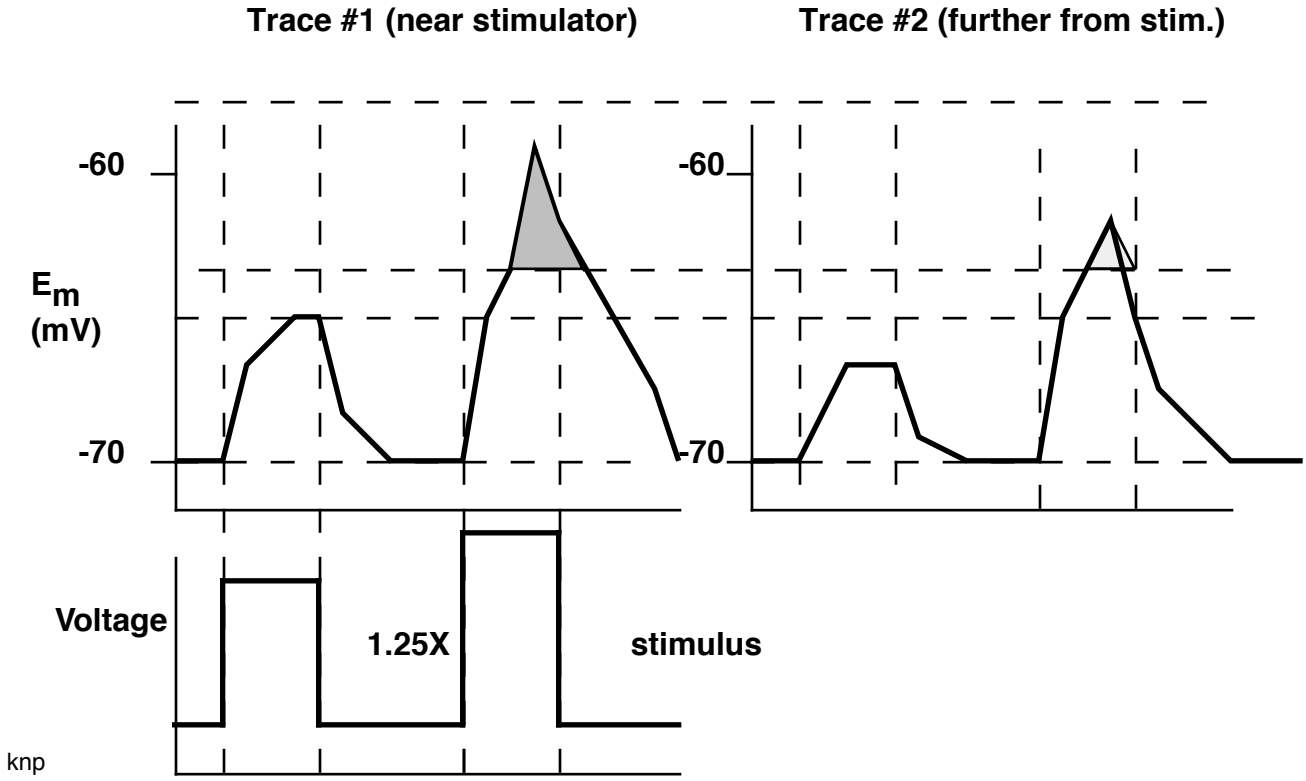
A. We have just completed a consideration of the electrotonic response. What if we continue to increase the strength of the stimulus? We will continue to employ the same experimental apparatus where we stimulate an axon and then record the results at 2 locations.

We will continue to use larger and larger levels of stimulation. The first two levels used will both be greater than the levels used previously; in the hypothetical example below the second is twice as great as the first and both are larger than those used above:

B. The first experiment of the day is given on the top of the next page:

Notice that there continues to be a relationship between (i) the stimulus strength and the size of the electrotonic response; (ii) an inverse relationship between the size of the response and the distance from the stimulus site; (iii) a shape that suggests an R-C type circuit, as in our model previously.

C. We now increase the strength of the stimulus even more. This time the first stimulus is greater than those shown above while the second is just slightly greater again:



1. Once again, the larger the stimulation, the larger the response. Thus, just like the electrotonic response, these responses are **GRADED RESPONSES**.

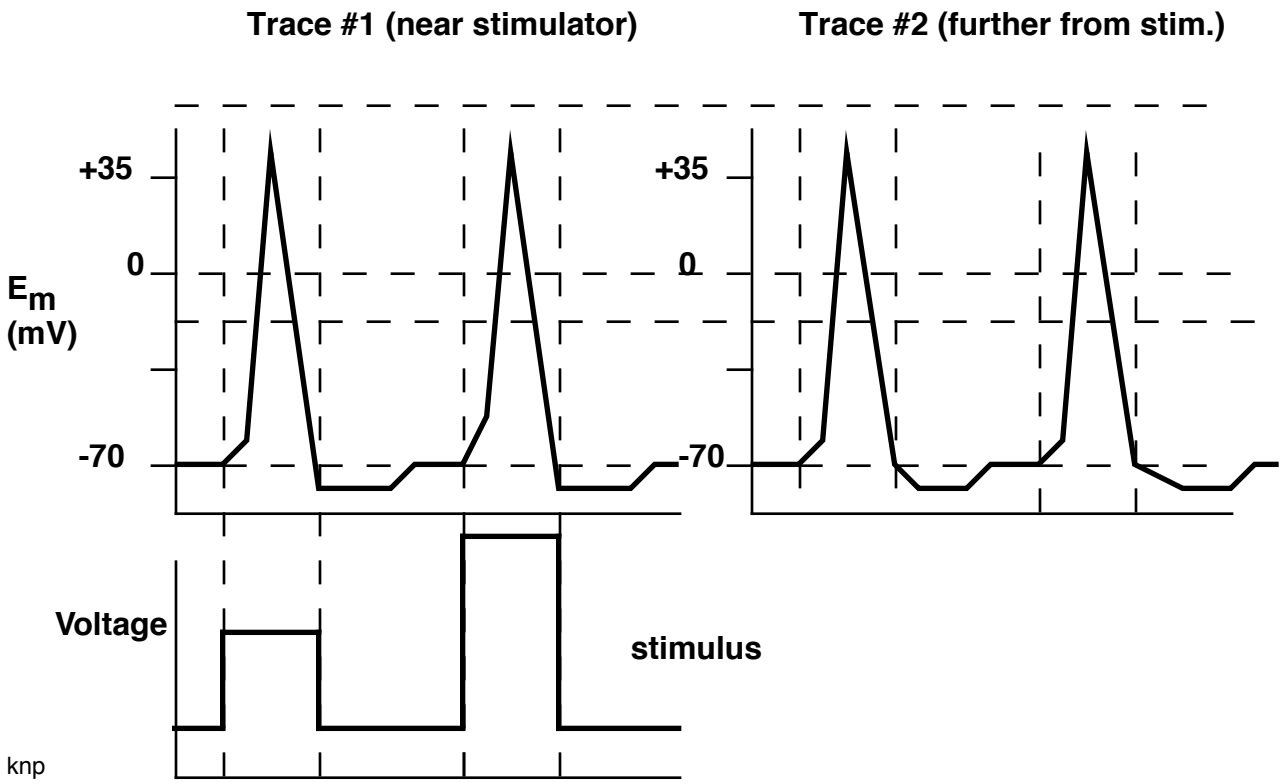
2. However, this time the second response is larger than would be predicted from a purely electrotonic response. (How large should the responses be if the response was purely electrotonic?)

3. The responses still die out with distance.

4. We call the second response, the one that is greater than predicted, a **LOCAL POTENTIAL**. The excess response over that predicted by the purely passive (electrotonic) model is referred to as an **ACTIVE RESPONSE**. In the diagram above the active response portion the response is shown as a stippled area as compared to the electrotonic response.

-- More about local responses later --

D. We now stimulate at even higher levels (these stimuli are much larger than those first discussed:



1. Now with only a slight increase in stimulus over the last one used a much larger potential results. Furthermore, the potential produced by the first stimulus is identical to the potential elicited by a much larger (2X the first) stimulus. **The response is not graded but is said to be "ALL OR NONE".**

2. The response does not die out with distance. In other words, it is **"conducted without decrement"**. We will show later that the response is actually **"self-regenerating"** -- that is, it is being produced all down the axon and its transmission is more analogous to a burning fuse than to electrical conduction.

3. These responses are called **ACTION POTENTIALS (APs)**

E. All three types of responses that we have seen are important in the study of the function of excitable cells. More on each of them later.

HERE IS A SUMMARY OF THE PROPERTIES OF LOCAL AND ALL-OR-NONE RESPONSES:

Graded	**All-or-None**
change in E_m is roughly proportional to stimulus intensity	same for all adequate stimuli
no threshold	threshold
no refractory period, can summate	refractory period
non-regenerative, decays over distance	regenerative
can happen anywhere on a neuron	axon only

! NOTES: **Threshold (b) is the minimum stimulus necessary to elicit a response.** More about this later.

Refractory periods (c) will be covered later, suffice it to say that **they are times when a second stimulus results in no response by the cell**.

V. THE VOLTAGE CLAMP AND THE STUDY OF MEMBRANE POTENTIALS

A. History

1. The events that occur during an AP were first elucidated for one type of cell by two British workers in the early 1950s, **Hodgkin and Huxley**. In many ways this discovery is at least as important as the discovery of the structure of DNA that occurred soon after.

2. Hodgkin and Huxley's success was in large part due to use of right organism (pointing up the vital importance of comparative studies) and the right technique.

a. They used the GIANT AXON FROM SQUIDS (not giant squid's axons -- too hard and dangerous to find!). Squids (and other cephalopods) contain several long axons that run the length of their mantle (remember this term from Bio 32?). These axons are very thick, often over 1 mm in diameter. (They are used to coordinate escape responses in the squid -- but not well enough, I guess, to avoid the collector's net!)

b. The technique Hodgkin and Huxley used is called the **VOLTAGE CLAMP**; we will spend most of this class considering it. In short, **it is a method that allows the investigator to see which direction ions flow relative to the cell during the AP. By combining the use of the voltage clamp with methods that either block the movement of certain ions (or by actually removing these ions), it is possible to see:**

1. which ions are moving and

2. to determine the direction and rate of their movements during an AP.

B. THE VOLTAGE CLAMP

1. **Definition: a voltage clamp is a device that keeps membrane voltage constant (i.e., it clamps the E_m) regardless of ionic movement.** By doing this, it is possible to determine what ionic currents are flowing through the membrane and furthermore, see how intense they are.

2. Reasons for needing a voltage clamp:

a. As we have pointed out earlier, when the E_m changes, flows of ions (CURRENTS) are involved. For example, we would predict from the Donnan equilibrium model that if a resting cell is stimulated to become more (+) on the inside (E_m approaches 0), this change in E_m is resisted by a K^+ current leaving the cell and a Cl^- current entering the cell; these currents attempt to re-establish the membrane at the equilibrium Em.

b. It was known that during an AP the membrane rapidly changed permeability to different ions and as a result many ionic currents produce the overall electrical event we call the AP.

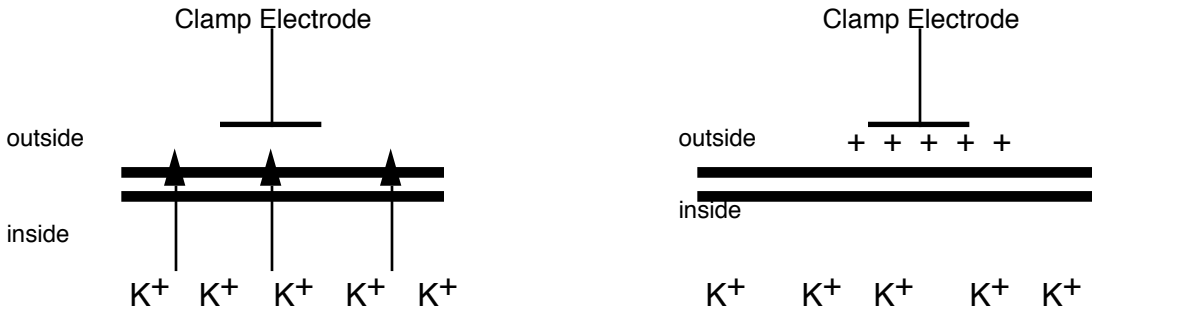
1. The question was: **which ionic currents flowed when and at what magnitude?**

2. What was needed was a device that would allow the quantification of the direction and magnitude of ionic current flows.

3. As we will see below, the voltage clamp allowed the quantification of the magnitude and direction of the ionic currents. When used in situations where one or more ions were absent, the changes in current could be used to determine which components were due to which ions.

a. The voltage clamp is a device that senses the E_m at any instant in time and then injects enough current to prevent the E_m from changing.

b. The amount of (injected) current needed to prevent a change in E_m ("stabilize" E_m) is equal to the size of the ionic flow. Furthermore, to stop the flow this current is applied in a direction opposite to the ionic flow.

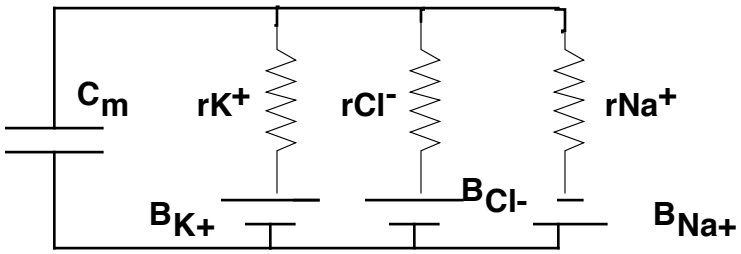


knp

Assume that for both situations conditions are such that K^+ movement out of the cell is favored. In the first case, if the electrode is off, the K^+ diffuses out down the electrochemical gradient creating a certain current, i_{K+}. In the second case, a current is applied by the electrode that is equal and opposite to i_{K+} and there is no net outward movement of K^+.

2. **Electrical properties of the membrane and the theory of operation of the voltage clamp:**

a. Be reminded of our earlier electrical model of the axon. We saw that there are both resistance and capacitance components to the membrane.



knp

b. We are concerned with changes in membrane current since the movement of ions must be the cause of the swings in E_m during and AP just as it is the cause of the resting potential.

c. From the last drawing in above, it is obvious that there are two **general paths for current to move** along.

1. One is through the **membrane resistances**, R_{K+}, R_{Cl-} and R_{Na+}.
2. The other is **into or out of the membrane capacitance**, C.

d. We call these two currents ***i_r* and *i_c***, respectively. The **total current, i.e., the membrane current *I_m* that can move through the membrane** is the sum of these two:

1. $i_m = i_r + i_c$

e. In the last class we learned that for the charge on a capacitor (in this case that charge causes the E_m) to change, the voltage across the capacitor (the E_m) must change. Thus, we can re-write the equation relating total charge to voltage and capacitance ($Q = C*E$) as:

2. $i_c = \frac{dV}{dt} * C_m$

where dV/dt is the rate of change in voltage of the membrane (for example, at any point during an AP) and C_m is the membrane capacitance. Note that we have replaced the static charge term Q with i_c, a moving charge term (i = current = charges/s). To do this, we had to also change the constant voltage term E to one of changing voltage dV/dt.

f. Since we now know the value of i_c, we can substitute it (from eq. 2) into eq. 1:

3. $i_m = i_r + \frac{dV}{dt} * C_m$

! In terms of a real membrane that is producing an action potential, **What do *dV/dt* and i_m correspond to?** (look at the figures of the action potential for a hint)

ANS: *dV/dt* is the rate of change of E_m at any point in time during the action potential. Im is the total ionic current moving across the membrane during these voltage changes.

!! BE SURE YOU UNDERSTAND THIS !!

g. In order to prevent E_m from changing, then the following condition must be met:

4. $i_m = i_r$

(or put another way this will be true if there is no change in E_m since $dV/dt = 0$.)

h. Thus to hold the E_m constant, we must apply a current that is equal to the current flowing through the membrane resistance (i.e., equal to the current flowing through the various ion channels). This current stops the ionic flow and prevents a voltage change.

DON'T PANIC IF YOU DON'T UNDERSTAND ALL OF THIS YET. WE ARE GOING TO LOOK AT SOME REAL VOLTAGE CLAMP DATA, AND THEN GO BACK AND OVER-VIEW ALL OF THIS. JUST BE PATIENT AND KEEP DOING YOUR BEST TO UNDERSTAND. SUDDENLY, IT'LL ALL COME LIKE A FLASH.

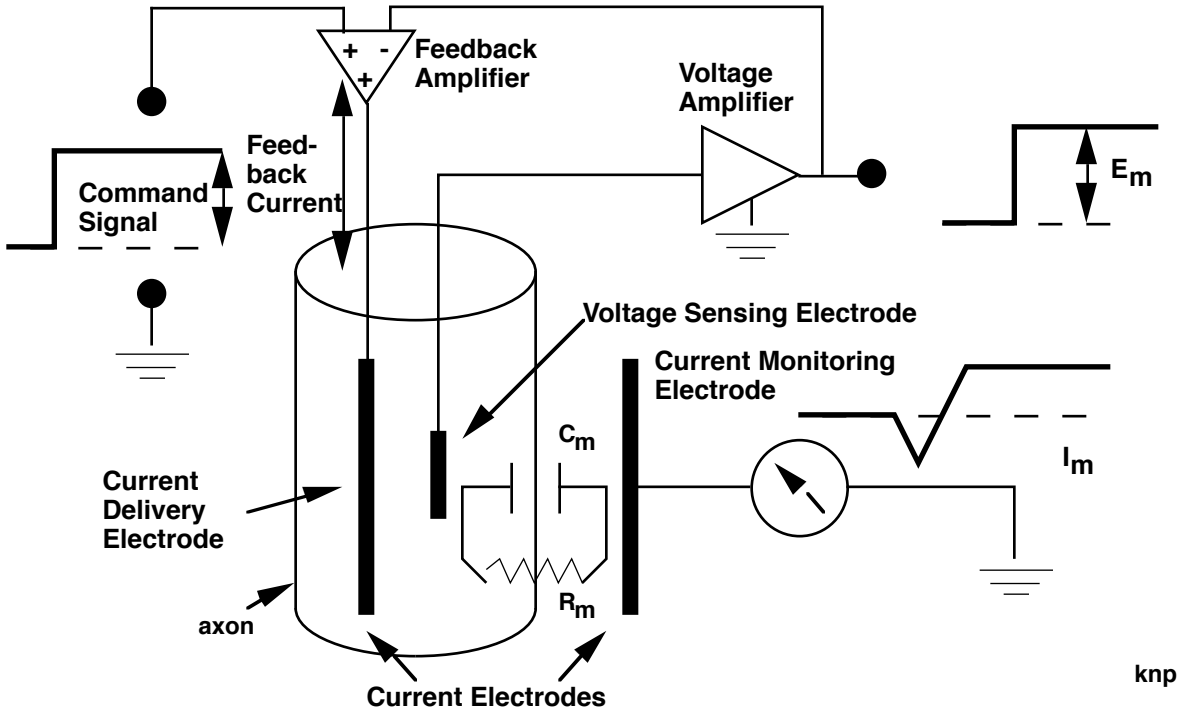
VI. VOLTAGE CLAMP EXPERIMENTS: THE NATURE OF THE ACTION POTENTIAL AND GRADED RESPONSES

A. The Action Potential:

1. We will consider voltage clamp experiments performed by Hodgkin and Huxley in the late 1940s and early 1950s. These data will be for the squid giant axon.

2. Experimental apparatus: The axon was removed and placed in a water bath where the ion composition and temperature could be controlled. A clamping electrode (current source)

was run down the middle of the axon so that current could be applied evenly down the length of the axon.



The Voltage Clamp. The COMMAND SIGNAL represents the value that the investigator has decided to set the membrane potential at (see upper left). Meanwhile, a VOLTAGE SENSING ELECTRODE located inside the cell senses the actual transmembrane potential, E_m. This value is amplified (upper right) and then sent to the FEEDBACK AMPLIFIER which detects any difference between the command voltage and the actual E_m. It then applies a current (the FEEDBACK CURRENT) between the CURRENT ELECTRODES that is of the correct magnitude and direction to keep E_m at the command value. This current is monitored at the external current electrode (see meter, lower right).

3. Notice that the experimenter decides the voltage at which to clamp the membrane. This value (upper left of diagram) is sent as a **command signal** to a feedback amplifier (triangle, top of fig). The output of this amplifier is the current that flows to the delivery electrode running through the center of the axon.

4. Another intracellular electrode senses the E_m , this is called the voltage-sensing electrode. This voltage is amplified by another amplifier (top right triangle) and becomes an input into the feedback amplifier. If the measured E_m is different from the command E_m decided by the experimenter, then the amplifier injects the appropriate current to bring actual E_m to command E_m.

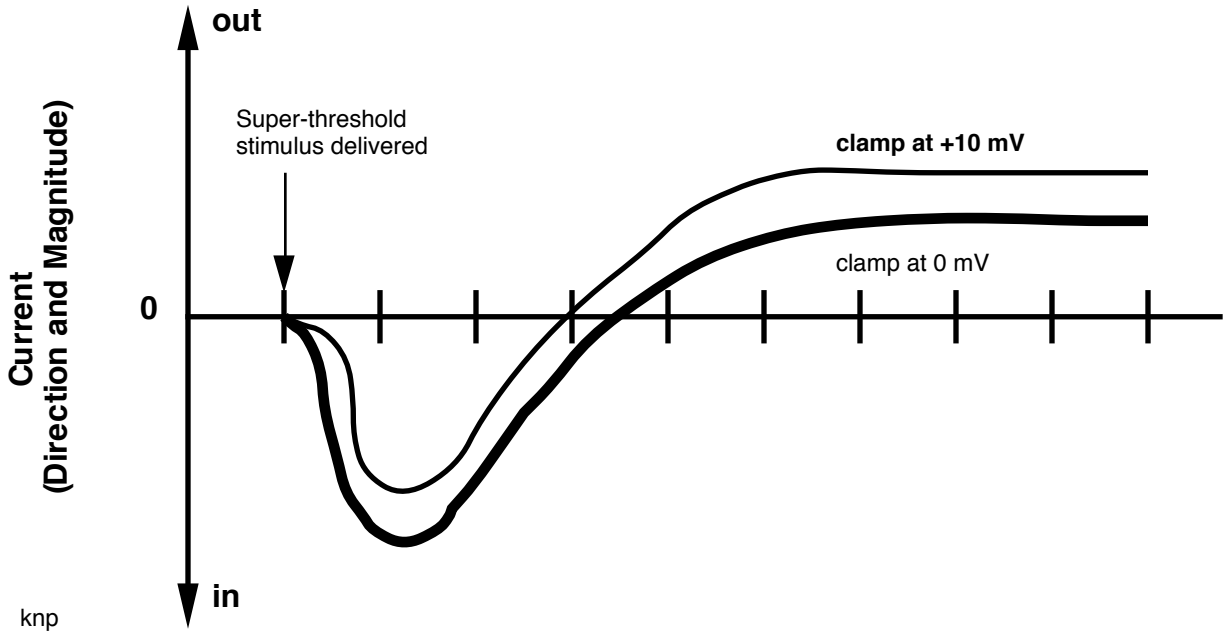
?: What type of feedback is the voltage clamp based on? Explain.

5. Finally, a current monitoring electrode, placed outside the cell measures the actual electrical current flow and its direction ((+) or (-)). **This current is the experimental output, and, as we have shown above, it is equal and opposite to any ionic currents that would tend to change the E_m from the clamped value.**

6. **Experiment**: Suppose we clamp an axon in a normal extra-cellular solution (containing K^+ and Na^+) at 0 mV (**assume that the normal rmp for the squid axon is -90mV)**. Also shown is a second experiment where the voltage is clamped at +10 mV.

? If the normal squid rmp is -90mV and membrane is moved to 0 or +10 mV but was not clamped, what would normally happen to E_m? Draw a graph of E_m with respect to time for these two cases in an unclamped membrane

Here are the experimental results of the voltage clamp experiment (note that directions are in terms of the ways that ions are moving):



knp

With the onset of the clamp (arrow) there is an immediate and increasingly more powerful inward current, which after a short time lessens and eventually becomes an extended outward current. Furthermore, the patterns are the same regardless of the degree to which the membrane has been depolarized from resting E_m. (However, remember that both of these clamped values are a long way from the normal resting E_m of -70 mV. Note that the voltage that we clamped the membrane at for this experiment is normally sufficient to elicit an AP.)

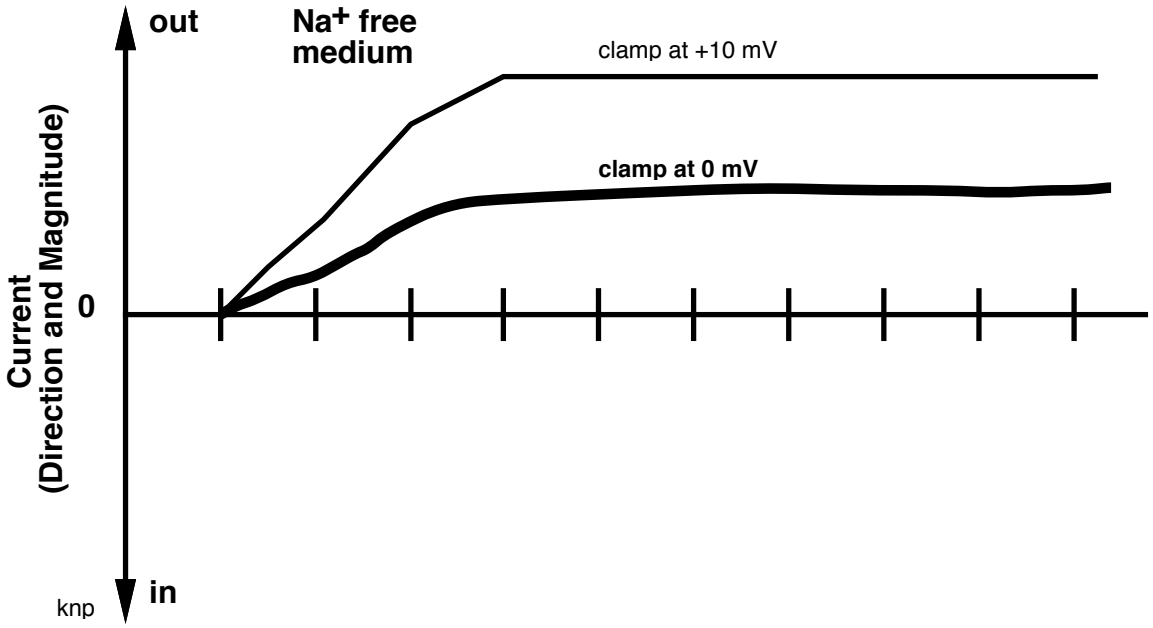
7. We now know the direction and magnitude of the overall ionic current with respect to time under normal conditions.

a. In order to identify which ions are involved in producing the AP, we will now do the experiment again EXCEPT THAT THE EXTERNAL [Na^+] = INTERNAL [Na^+]. THUS THERE IS NO GRADIENT FOR Na^+ to enter or leave the cell and it will not diffuse.

b. In order to prevent the cell from having osmotic changes due to a hypotonic medium (since the Na^+ has been removed to make it as low as the internal Na^+), Na^+ is replaced with a non-diffusible, non-toxic (+) ion, **choline**.

c. K^+ and Cl^- are still present in normal extra-cellular concentrations.

Here is the same experiment:



Notice that there is now no inward current -- only a outward current appears and it eventually will intersect the curve for the first set of experiments.

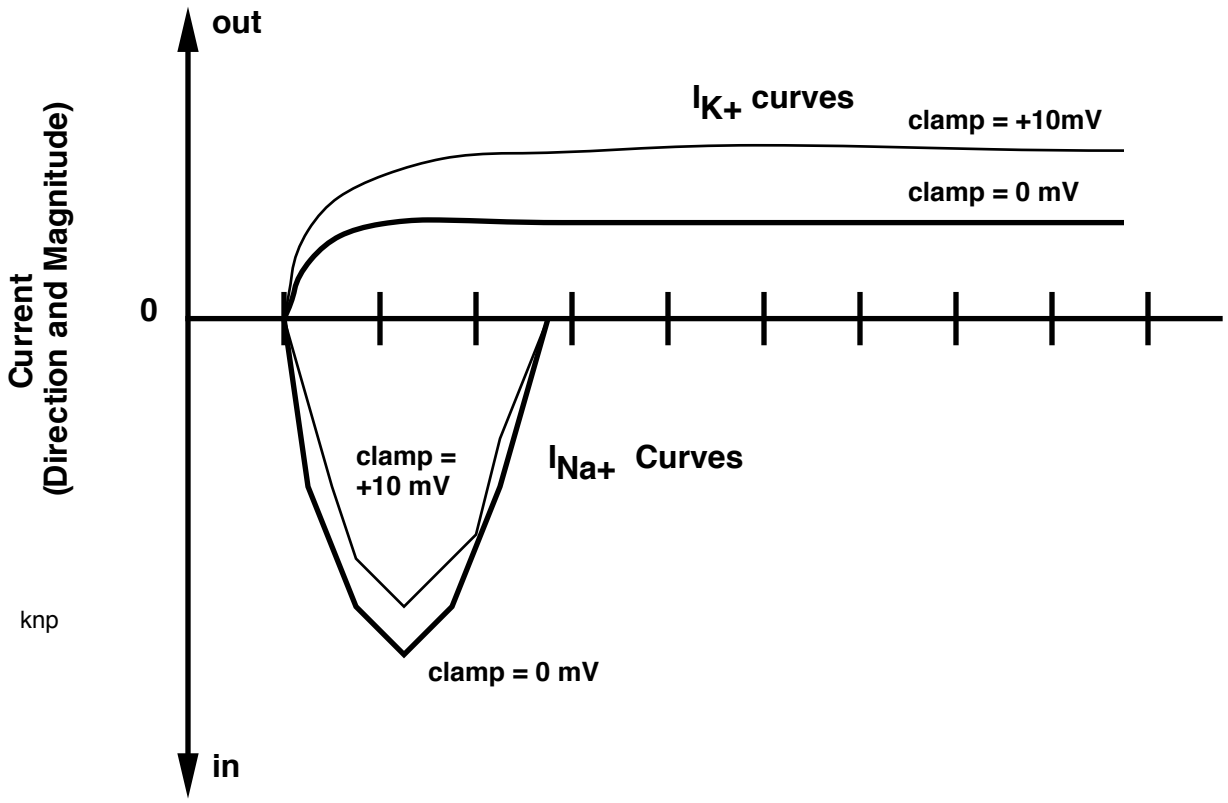
9. Thus, it is obvious that **the inward current observed in experiment 1 is carried entirely by Na^+ -- removal of Na^+ completely eliminates the inward current.**

10. **We can now use the data from experiments 1 and 2 to determine the real values of the Na^+ and Cl^- flows.**

a. By **subtracting the curve obtained in experiment #2 (Na^+ removed) from the curve of experiment #1 (all ions present)**, we get the curve for **<u>Na^+ current,</u> i_{Na+}.**

b. The curve obtained in experiment #2 is the K^+ (and Cl^-, but we ignore that at present) curve -- the reason is that for the same E_m conditions as in the first clamp experiment there is now no Na^+ and so the only ion that moves is K^+. NOTE: Cl^- doesn't matter, for the same reasons as were already discussed in regards to the resting E_m -- Cl^- follows passively what the (+) ions do.

c. Thus, the two separate currents (with AP above for a reference) look like this for our squid axon (next page):



d. We can now partially explain the events of an action potential. A more complete explanation will be made in the next class.

1. The **rapid depolarization of the AP is due to rapid entry of Na^+ into the axon. The rate of Na^+ entry is greatest near the middle of the depolarization; it decreases rapidly after that**.

a. Na^+ is entering the cell in response to both a diffusion gradient and an electrical gradient.

b. As Na^+ enters the cell (going down its electrochemical gradient), it brings in (+) charges and makes the cell more and more positive.

c. Since Na^+ cannot normally enter the cell at such high rates, we must posit the existence of **GATED Na^+ CHANNELS**: proteins that can, under certain conditions allow Na^+ to enter the cell at very high rates (the gates open).

f. Thus, the **PERMEABILITY OF THE CELL TO Na^+ HAS INCREASED.**

e. These gates only remain open for a certain period of time.

2. Late during the depolarization (also called the UPSWING), K^+ begins to leave the cell at higher than usual rates. K^+ continues to leave the cell at high rates during the subsequent **repolarization** of the cell to resting E_m.

a. K^+ is also moving down its electrochemical gradient since K^+ is already more concentrated inside than outside and furthermore, the inside is now (+) instead of (-).

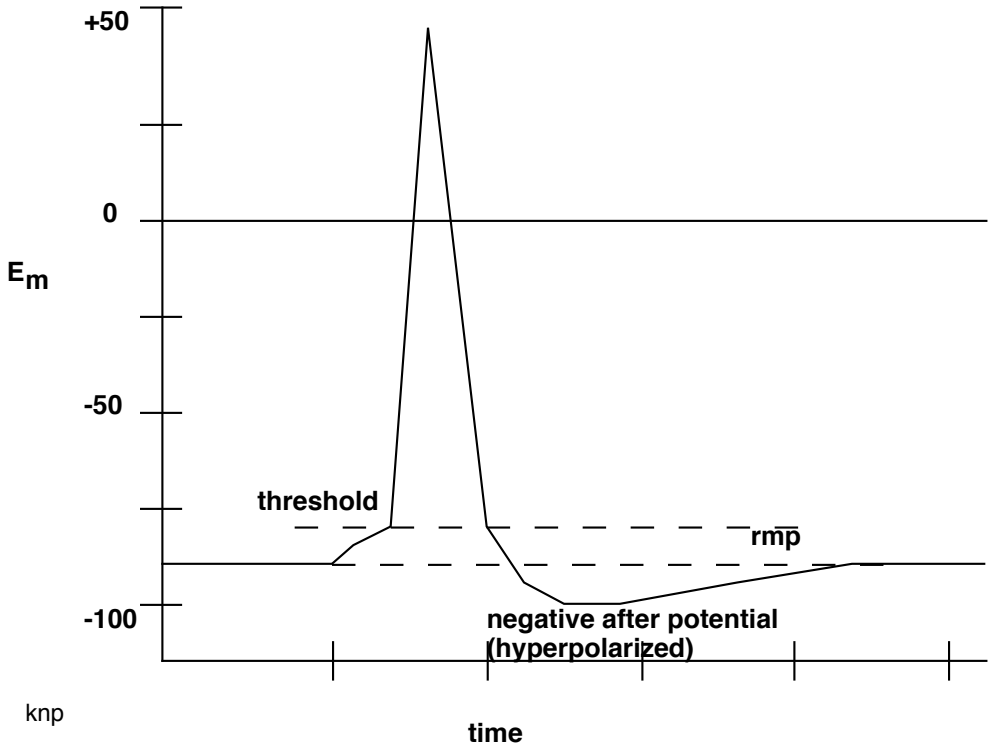
b. Each K^+ that diffuses outward carries a (+) charge with it. This leaves the inside more (-) and repolarizes the cell. Note that by the time the K^+ is leaving in high rates, Na^+ once again appears to be unable to enter the cell.

c. The period when the cell is repolarizing is called **RECTIFICATION**.

d. As with the case of Na^+ it appears that the cell has become more permeable to K^+ during the rectification period.

e. Note that for a brief period of time during repolarization the E_m is more negative than the normal resting E_m. During this period of time the cell is **HYPERPOLARIZED**. More about this next class.

3. Cl^- moves passively to its correct electrochemical gradient in response to the Na^+ and K^+ events outlined above. However, be aware that at least in some cells, Cl^- permeability changes also occur.

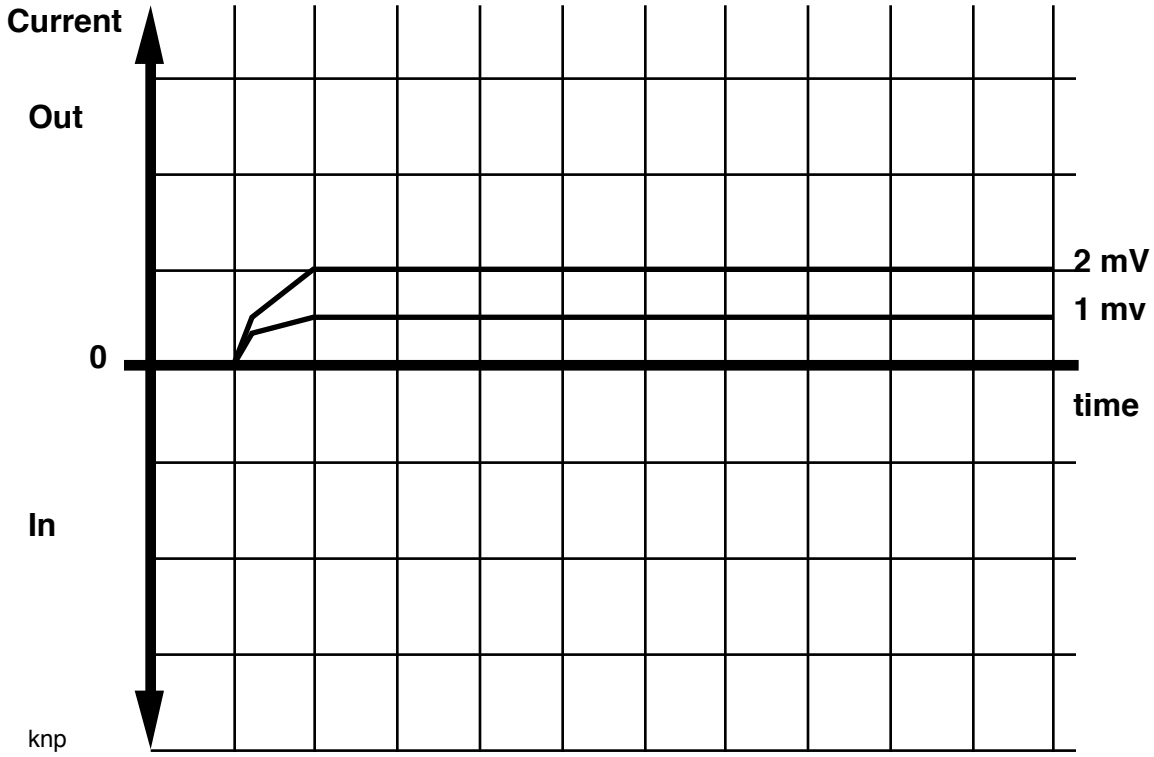


VII. Experiments where the clamp voltage is close to the normal E_m.

A. **ELECTROTONIC AND LOCAL RESPONSES AND THE VOLTAGE CLAMP**

1. Suppose that we clamp the membrane at a voltage just slightly more positive than the normal RMP -- that is, at a value identical to those that gave the electrotonic responses shown earlier.

Here are the results if the cell is placed in a normal medium. **The two curves are for a 1 and 2 mV depolarization** (i.e., from the resting E_m (RMP) of -70 mV to -69 or -68 mV)

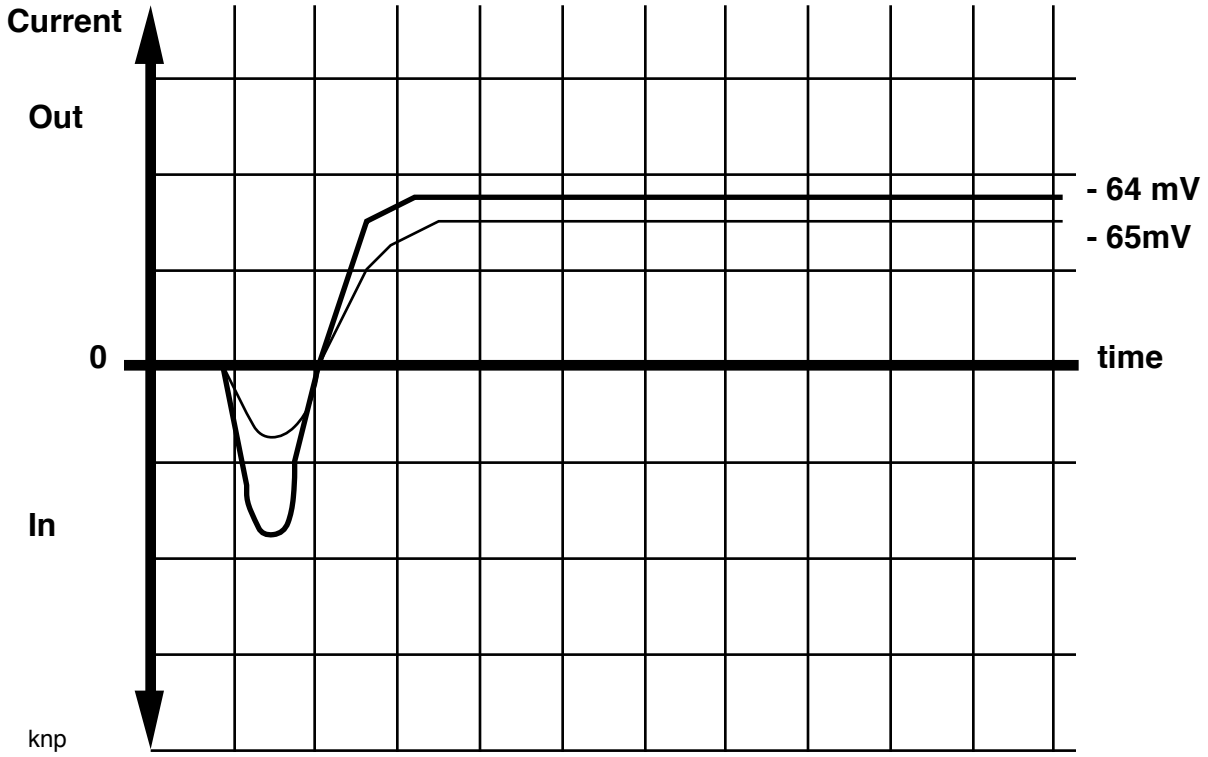


There is no evidence of any inward current

IDENTICAL RESULTS ARE OBTAINED IN AN ENVIRONMENT WHERE $[Na^+]_{in} = [Na^+]_{out}$

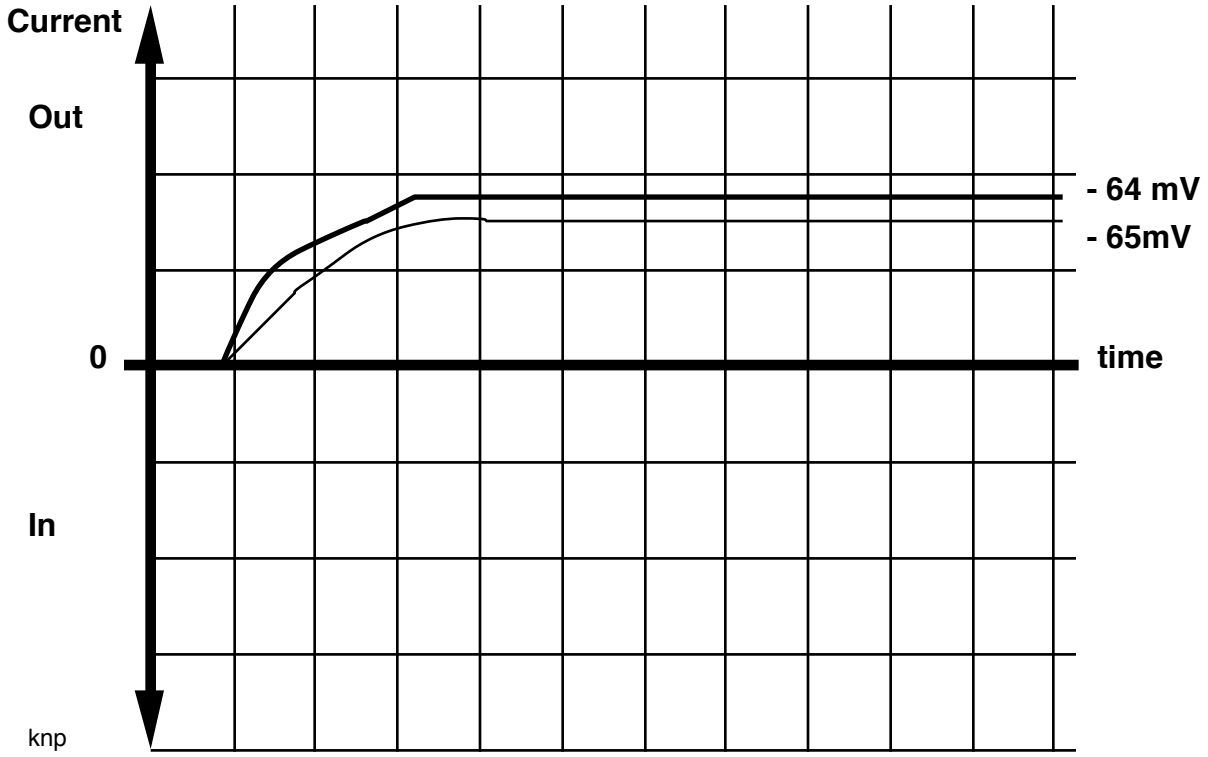
We will return to these results shortly.

2. Experiment #2. In this case the membrane is clamped at the two E_m values where we got local responses (these are described in the notes for class #6, page 1).
Thus, if the RMP was -70 mV, we will depolarize and clamp the membrane at -65 and -64 mV.
Once again the first experiment will involve the use of a normal extra-cellular solution.



A small inward current appears in these cases, unlike the previous experiment where no inward current was observed.

If we repeat the experiment where the $[Na^+]_i = [Na^+]_o$:



Notice that in this case there is no inward current.

3. Interpretation of these two experiments:

a. In the first experiment, there was no inward current, only an outward current appeared. Thus, at small depolarizations, no Na^+ entered the cell but K^+ did leave the cell and the rate of efflux of K^+ was proportional to the size of the depolarization.

1. Given our model from the end of the last class (page 8), it appears that during electrotonic responses no Na^+ can enter the cell because the Na^+ gates remain closed.

2. K^+ leaves the cell as long as the cell is depolarized in response to the lowered E_m. Under these conditions, there is a less favorable gradient to keep K^+ in the cell where it is highly concentrated (think of the Nernst eq. and what happened to the K^+ ratio if E_m is moved towards 0). Thus, the rate of efflux is greater with the greater depolarization.

! The K^+ response can be thought of as a response that tends to return the membrane towards the normal RMP. **WHY DOES IT DO THIS?**

b. In the second experiment, slight amounts of Na^+ entered but the explosive increase in Na^+ permeability seen in the data covered for the AP (previous set of notes) did not occur. Other important observations: much more Na^+ entered at the +6 mV depolarization than at +5 mV; this corresponds with the observations shown in the a previous packet where there was a big increase in local response between these two levels of stimulation. Lastly, note that once again there was a K^+ efflux from the cell that is proportional to the size of the depolarization.

1. The Na^+ entry signifies that some of the Na^+ gates have opened. Furthermore, the fact that the degree of opening of the gates appears to increase rapidly with a increase in depolarization suggests that their operation is **strongly voltage dependent. (based on the voltage clamp data for this class and last).**

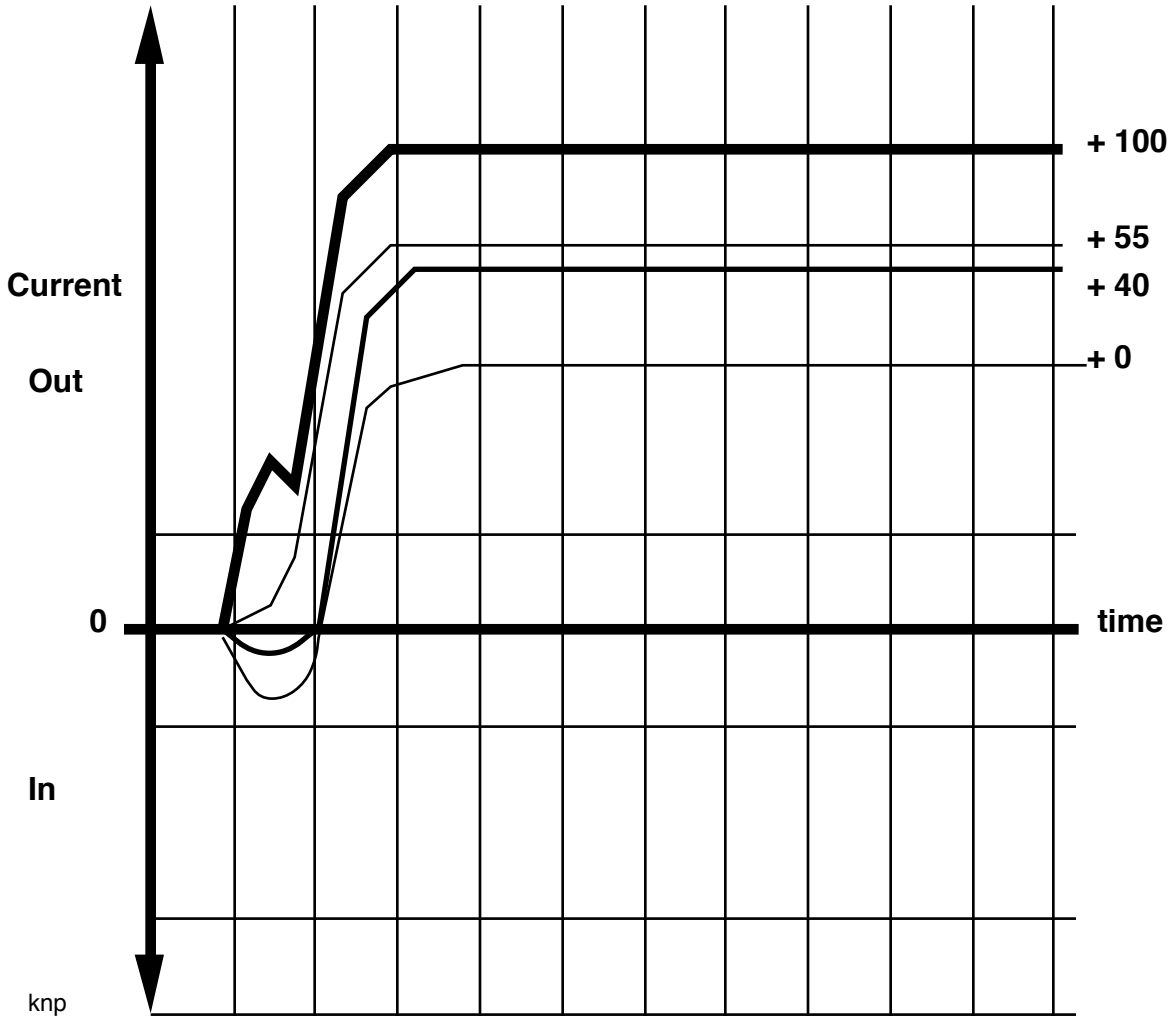
2. It also apparent that Na^+ gates are only opened for a short period of time.

3. The fact that K^+ efflux is once again proportional to the E_m is not surprising, for the same reasons as given above.

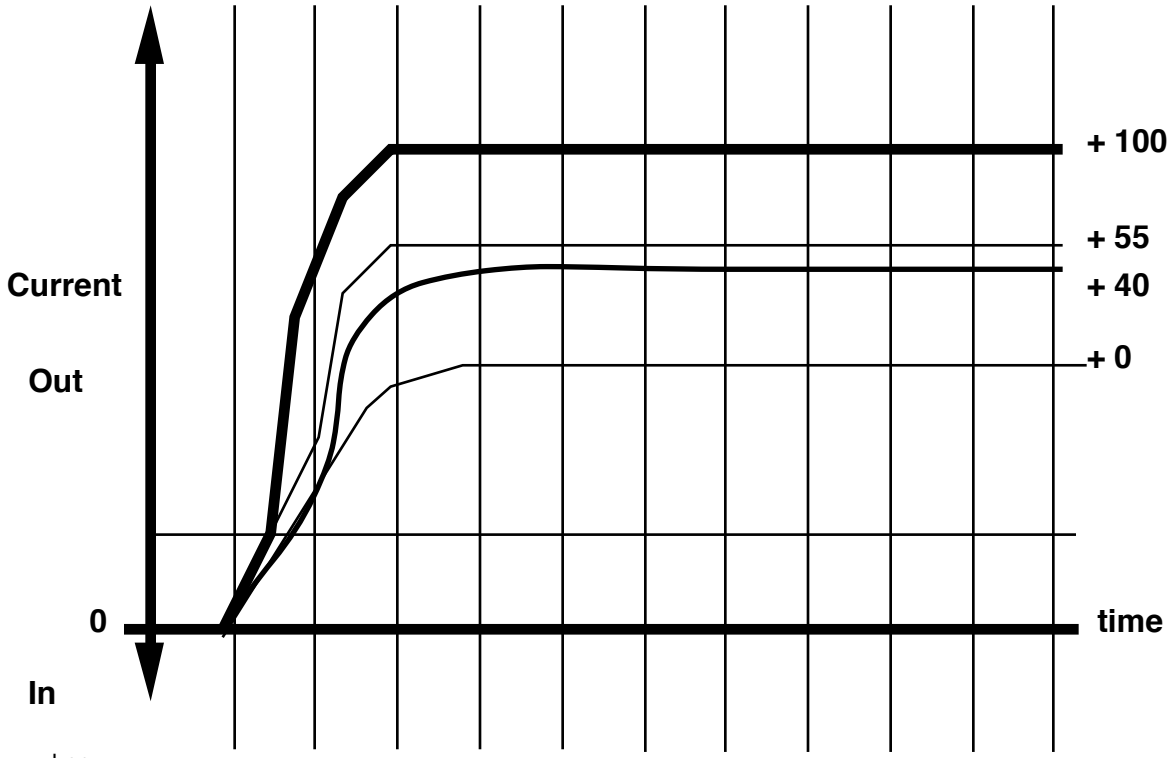
B. VOLTAGE CLAMPS AND HIGH (+) VALUES OF E_m

1. Let's look at one final experiment before we attempt to put together all of this information about E_m and changes.

a. We will put the squid axon in a normal extracellular fluid and clamp it at +0, +40, +55 mV and +100 mV:



Here are the results for the same clamp values but where $[Na^+]_i = [Na^+]_o$:



b. The new thing to see here is that at high clamp voltages (above 0 mv), the inward Na^+ current tends to disappear and at even higher voltages the Na^+ tends to flow out of the cell. As usual, the K^+ outward current tends to increase as the E_m is clamped at more and more (+) values.

2. Explanation:

a. As you may recall from the lectures on RMP, if Na^+ were the determining factor for the E_m, its value would be somewhere near +50 mV. This was referred to as the Na^+ equilibrium voltage just as we also calculated K^+ and Cl^- equilibrium voltages.

b. Thus, if we clamp the membrane at a voltage equal to the Na^+ equilibrium voltage there is no net force favoring the entry of Na^+ into the cell and so none enters even though the membrane may be quite permeable to Na^+. Of course, the exact same situation exists for Cl^- at the RMP where the E_m is equal to the Cl^- equilibrium potential and as we know, there is no net movement of Cl^- .

c. At clamp values above the Na^+ equilibrium potential, the net forces favor the movement of Na^+ out of the cell.

? Using the Na^+ data for the squid axon (earlier packet) and the Nernst equation, show that Na^+:
should neither enter nor leave the cell at a potential of +55 mV,
should leave the cell at +100 mV and
should enter the cell at 0 mV.

VIII. PERMEABILITY, CONDUCTANCE AND CURRENTS

A. We now have enough data to define a series of important characteristics of the membrane that will allow us to better understand excitability.

B. **PERMEABILITY (P_{ion}):** is simply **a measure of the ease with which a substance can pass through the membrane as the result of a concentration gradient.** It is an open-ended number running from 0 (no permeability) to infinity (no membrane!). The units of permeability are

$$\frac{\text{mols}}{(\text{cm2} * \text{s} * \text{cm})}$$

C. **CONDUCTANCE (G_{ion})**: This is concept very **similar to permeability** except that it is **defined in electrical** as compared to concentration (in the case of permeability) **terms** attempting to move across the membrane.

1. Remember that in earlier terms, we defined conductance as the reciprocal of resistance, that is:

5. $$G = \frac{1}{R}$$

2. Both conductance and permeability are largely properties of the membrane: it is the specific protein channels that exist in the membrane that determine how easily and under what conditions that an ion can enter or leave a cell.

D. **CURRENT (I_{ion}): Current is the actual flux ($\frac{\text{amount}}{\text{(area * time)}}$) of a certain ion.**

1. Mathematically the concept can be derived from Ohm's law and the definition of conductance:

6. $$I = \frac{E}{R} = E * G$$

7. $$I = E * G$$

For a specific ion:

8. $$I_{\text{ion}} = G_{\text{ion}}(E_{\text{M}} - E_{\text{ion}}) = G_{\text{ion}}\Delta E$$

where (E_m - E_{ion}), the electrochemical driving potential, is the difference between the membrane potential and the equilibrium potential for a given ion.

2. It should be obvious from eq. 8 that if the membrane conductance for a certain ion is low, the current of that ion will be low regardless of the gradients favoring entry into the cell. Likewise, even if conductance is high for a certain ion, if the E_m and equilibrium potentials are equal, there will be no current for this ion.

E. CHANNELS AND GATES:

1. We've been talking a lot about gates etc. without really saying what they are and if there is any real evidence for their existence.

2. **CHANNEL: a transmembrane protein that will allow one or more types of ions to pass.** Usually they are quite specific and allow only 1 or 2 types to pass. A good example is the Cl^- channel that permits Cl^- to go in either direction across the membrane in response to its electrochemical gradient.

3. GATED CHANNEL: a channel that only allows the specific ions to pass under certain conditions. Once they open, they often only remain open for a short period of time and then close. **Characteristics of gated channels**:

a. the **ion(s) that they are specific for.**

b. the **conditions under which they will open (usually the voltage required for their opening).**

c. their **KINETICS**:

i. **how long they open**

ii. **their refractory period: how long is it before they can open again after having opened** (think of this as time needed to reset the gated channel).

We will usually use the term "gated channel" synonymously with "channel" or "gate". What we really mean will be obvious from the context.

4. Some gated channels:

a. the Na^+ channel

b. K^+ channel**s**

c. Ca^{++}/Na^+ channel

F. IDENTIFICATION OF GATES:

1. usually **if there are time- or voltage-dependent kinetics involved in the movement of an ion across the membrane, the presence of a gate is usually necessary to explain this behavior**.

2. **PHARMACOLOGICAL BLOCKERS**: drugs that prevent certain ionic currents and that can be shown to bind to proteins on the membrane are good evidence for the presence of a gate. Important examples:

a. **Na^+ gate: TTX (tetrodotoxin**, from the puffer fish), **STX (saxotoxin**, from some shellfish).

b. **K^+ gates: tetra-ethylammonium (TEA)**

c. **Ca^{++}/Na^+ gate: Mg^{++}, verapamil**

IX. INTERPRETATION OF THE VOLTAGE CLAMP DATA: THE NATURE OF ELECTROTONIC, LOCAL AND ACTION POTENTIALS:

A. ELECTROTONIC RESPONSE:

1. If E_m is shifted away from E_{K+}, K^+ will begin leaving the cell as predicted by eq. 8, above. That explains the current leaving the cell that can be blocked by TEA. The magnitude of this current will be directly proportional to the degree of depolarization (also as predicted by eq. 8). When the stimulus is turned off, this K^+ current is responsible for repolarizing the membrane.

2. The shape of the electrotonic response is a result of the capacitive elements present in the cell, as was discussed earlier.

B. LOCAL RESPONSE:

1. the E_m is depolarized enough that some of the Na^+ channels open. As a result, E_m moves further towards zero than it would in purely electrotonic response. This is because G_{Na+} is higher than normal and according to the Goldman eq. Na^+ will partially determine E_m. (NOTE: A THE GOLDMAN EQ. CAN BE EXPRESSED WITH IONIC CURRENTS INSTEAD OF PERMEABILITIES --- YOU SHOULD RE-WRITE THE EQ. WITH THAT IN MIND).

2. With even greater depolarizations, more K^+ leaves the cell and repolarizes it when the stimulus ends.

3. Thus **the local response is part electrotonic response, part "active" response.**

C. ACTION POTENTIAL

1. We saw from the voltage clamp data that the Na^+ current increased rapidly from nothing to a very large but constant value over a very short range of stimulatory voltages. This has to do with the voltage sensitive properties of the Na^+ channel.

a. at E_m values that are more negative than a certain critical value, the Na^+ channels are unable to open.

b. there exists a degree of depolarization, called the **THRESHOLD** where a sufficient number of Na^+ channels will open to begin a rapid, almost explosive depolarization of the membrane. More about this in a moment.

2. **EVENTS**:

a. Assume a depolarizing stimulus is applied so rapidly that K^+ cannot leave rapidly enough to prevent E_m from changing.

b. As soon as the E_m approaches or equals or the threshold, Na^+ channels start to open and G_{Na+} increases. Since Na^+ enters bearing a (+) charge, the membrane is further depolarized.

c. This further depolarization, induced by entering Na^+, causes more Na^+ channels to open and more Na^+ enters further depolarizing the membrane. We now have a positive feedback situation operating.

d. Thus, the membrane *dV/dt* increases rapidly as more and more Na^+ enters the cell. Na^+ is entering much more rapidly than K^+ can leave because:

1. during the first half of the upswing the electrochemical gradient for Na^+ is much more favorable than that of K^+

2. with most of the Na^+ channels open, the membrane is much more permeable to Na^+ than to K^+, a reversal of the usual situation.

3. The Na^+ current increases as the channels open up to a point – as more and more open and as Na^+ enters the cell depolarizing it, E_M begins to approach E_{Na^+} and there is less of a driving force for Na^+ entry – i_{Na+} begins to decrease by late in the upswing phase of the AP.

4. G_{Na+} reaches it's maximum at the peak of the AP, but by this point i_{Na+} is quite small since, as just stated E_M and E_{Na^+} are now very close to each other and there is little driving force for Na^+ entry (even though G_{Na+} is high).

? What are the approximate relative values of the depolarizing (Na^+) current and combined

repolarizing currents (K^+ and Cl^-) at the peak of the AP?
ANS: they are very close to being equal – WHY?

e. To end the AP and return to E_M the cell must restore resting membrane conductances; ion movements to repolarize the cell will closely follow the changes in conductance.

1. Na^+ gate inactivation – there are far more voltage-gated Na^+ channels than any other type of ion channel and these need to close. They do so by self-inactivation. The maximum number of gates is opened by the top of the upswing and by the time the membrane is repolarized, the voltage gated channels are all closed.

? Use the Goldman equation (previous set of notes) to show why the shape of the AP must largely mirror the changes in G_{Na+}.

2. Also very important: the Na^+ channels now must remain controlled for a set period of time before it is possible for them to open again. This assures that repolarization will occur. The period of time when Na^+ channels will not open is referred to as their **REFRACTORY PERIOD**.

f. **RECTIFICATION** occurs because:

1. the E_m is a long way from the K^+ equilibrium potential and thus relatively large movements of K^+ is favored.

2. an additional, voltage sensitive population of K^+ channels opens that increases G_{K+} to values that are even greater than the normally high values found at rest.

a. we know these channels must exist because we cannot account for all of the increase in I_{K+} seen during rectification entirely as a result of a greater electrochemical gradient (eq. 8).

b. these slow K^+ channels have different kinetics and voltage sensitivity than do the Na^+ channels: they come into operation only at very (+) E_m values and remain open for a long period of time.

3. As a result K^+ leaves the cell in greater than normal amounts, thereby repolarizing it.

4. Note that for a short period of time the E_m actually becomes more (-) than normal, that is, it is **HYPERPOLARIZED**. This is due to the abnormally high G_{K+} at this point: the slow K^+ channels are still opened (although closing). Thus, E_m moves more towards the E_{K+} that we learned earlier is more negative than the actual E_m.

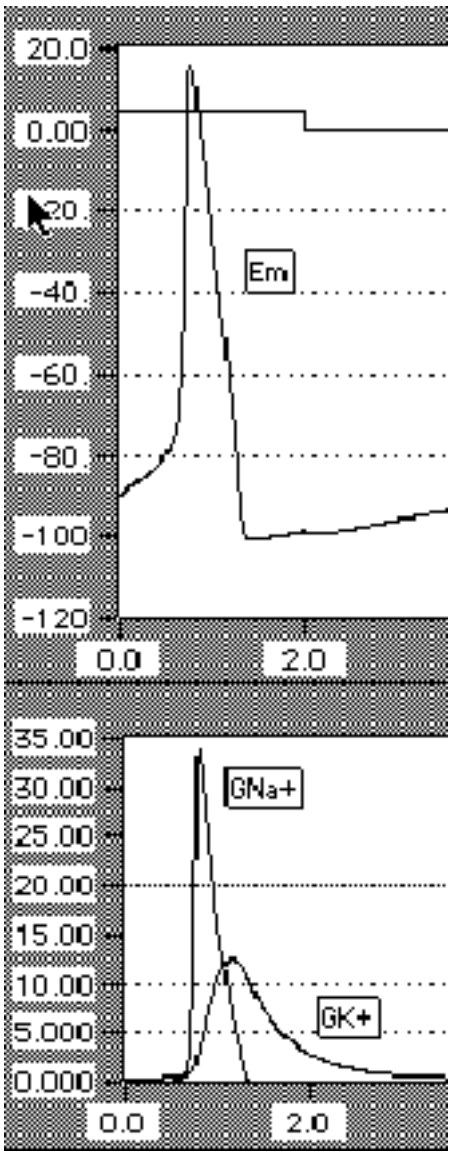
5. Finally as G_{K+} approaches its normal values, E_m returns to normal.

3. Blocking the pump has no effect on the depolarization-repolarization events.

NOTE THAT ALL OF THESE EVENTS TOOK PLACE WITHOUT THE ACTION OF THE Na^+/K^+ ATPase. THEY ARE ENTIRELY THE RESULT OF DONNAN-ASSOCIATED PROPERTIES AND THE CHANGES IN MEMBRANE CONDUCTANCE.

? If we treated a neuron with ouabain and then stimulated it over a long time period, what would happen to E_m? Why? What does this indicate about the importance of the Na^+ pump?

4. We can summarize the events of the AP showing both the AP and the G_{ions}. Note that the G_{ions} values are derived from the conductances from voltage clamp experiments and then calculated using eq. 4. You should be able to do this from the raw data. (what is shown on the next page is from a simulation that you will use next week to review the AP).



5. Looking at these curves, we can now define three terms that are important in excitable cell physiology:

a. **THRESHOLD**: the minimum change in E_m necessary to induce a sufficient number of Na^+ channels to open to get a rapid depolarization and action potential. (or more simply, **the minimum stimulus required to elicit an AP**.)

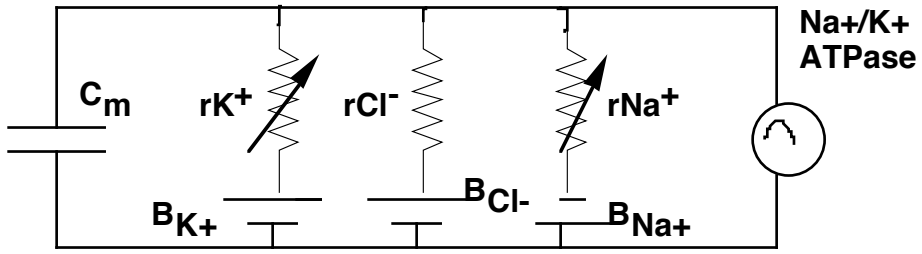
b. **ABSOLUTE REFRACTORY PERIOD (ARP)**: **the period of time when a second stimulus, no matter how large, cannot elicit a second AP**. It is due to the inability of the Na^+ channels to reopen.

c. **RELATIVE REFRACTORY PERIOD (RRP): the period of time following the ARP when a second stimulus can elicit an AP if it is larger than the normal minimal stimulus required to elicit an AP.** It is due to the fact that during the RRP the membrane is hyperpolarized and a larger than normal stimulus is required to get the cell to threshold.

6. Given what we know about the changes in G_{ions} during the AP, we must now update our membrane model. The principal difference of this model from the last is that we have replaced the fixed resistances for Na^+ and K^+ with variable resistances that indicate that the permeability of the membrane for these ions changes with time

? Why don't we change to variable batteries also?

The model:



knp

Now to tie all of this up, you should be able to answer the following: (I EXPECT YOU TO BE READY TO DO THIS!)

1. Discuss the membrane model and the events of an AP.
2. Discuss the relationship between the different variables in the Goldman eq. and the events of the AP.
3. Why do only a few Na^+ channels open in the local response while many open in the AP?
4. Draw a diagram showing the # of Na^+ channels with respect to time during an AP (draw an AP with it for comparison). See the last couple of pages and the Goldman equation for some ideas!
5. Do you expect the E_m to actually reach the Na^+ equilibrium potential during an AP? Why?
6. Regarding smooth muscle APs -- APs in these cells are usually produced by an influx of Ca^{++}. The ratio of Ca^{++} across a membrane is usually not as great as Na^+. What does this and the Nernst Eq. tell you about the magnitude of the Ca^{++} "spikes" (APs) produced by these cells when compared to the Na^+ spikes of neurons and skeletal muscle cells?
7. Write down one or two questions of your own, we'll try them.