The Nerve Impulse
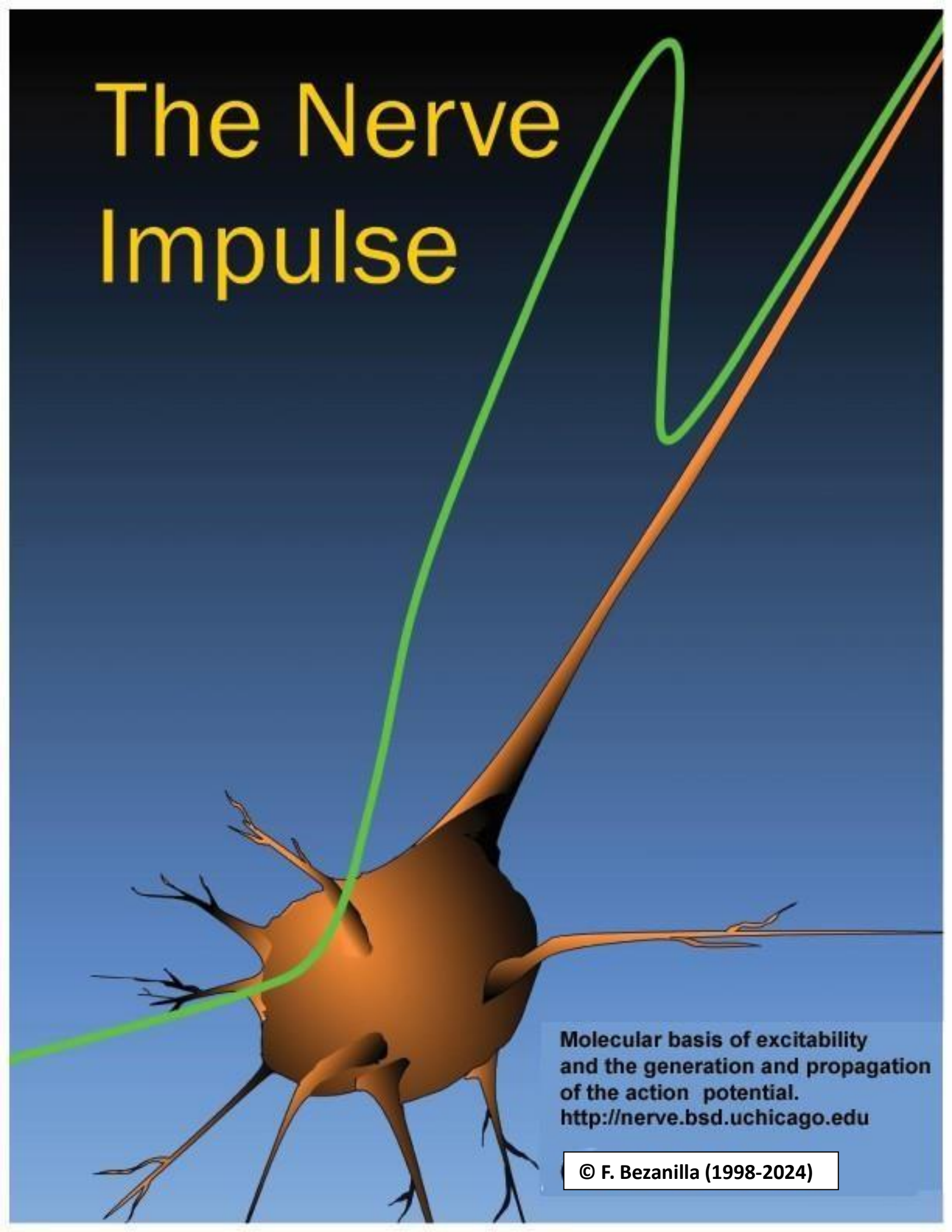
Molecular basis of excitability
and the generation and propagation
of the action potential.
http://nerve.bsd.uchicago.edu

The Nerve Impulse.

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Contents

INTRODUCTION

Axons are responsible for the transmission of information between different points of the nervous system and their function is analogous to the wires that connect different points in an electric circuit. However, this analogy cannot be pushed very far. In an electrical circuit the wire maintains both ends at the same electrical potential when it is a perfect conductor or it allows the passage of an electron current when it has electrical resistance. As we will see in these lectures, the axon, as it is part of a cell, separates its internal medium from the external medium with the plasma membrane and the signal conducted along the axon is a transient potential difference[1] that appears *across* this membrane. This

potential difference, or *membrane potential,* is the result of ionic gradients due to ionic concentration differences across the membrane and it is modified by ionic flow that produces ionic currents perpendicular to the membrane. These ionic currents give rise in turn to longitudinal currents closing local ionic current circuits that allow the regeneration of the membrane potential changes in a different region of the axon. This process is a true propagation instead of the conduction phenomenon occurring in wires. To understand this propagation we will study the electrical properties of axons, which include a description of the electrical properties of the membrane and how this membrane works in the cylindrical geometry of the axon.

Much of our understanding of the ionic mechanisms responsible for the initiation and propagation of the action potential (AP) comes from studies on the squid giant axon by A. L. Hodgkin and A. F. Huxley in 1952. The giant axon has a diameter in excess of 0.5 mm, allowing the introduction of electrodes and change of solutions in the internal medium. These studies have general relevance because the properties of the squid axon are very similar to non-myelinated nerves in other invertebrates and vertebrates, including man.

The Capacitance and Resistance of the membrane.

The plasma membrane is made of a molecular lipid bilayer. Inserted in this bilayer, there are membrane proteins that have the important function of transporting materials across the membrane. The lipid bilayer acts like an insulator separating two conducting media: the external medium of the axon and the internal medium or axoplasm. This geometry constitutes an electric capacitor[2] where the two conducting plates are the ionic media and the membrane is the dielectric. The capacitance c of a capacitor increases with the area of the plates and decreases with the separation between the plates according to the the relation

$$c = \epsilon \frac{A}{d} \qquad (1)$$

where A is the membrane area, d is the separation between the plates or the membrane thickness and is the dielectric constant. In the case of the membrane, it is more convenient to define the capacitance as independent of the amount of area involved and call it the specific capacitance C_m which is defined as the capacitance per unit area *or c/A*. Replacing this definition in eq (1) we find

$$C_m = \frac{\epsilon}{d} \qquad (2)$$

As the thickness d is only 25 A, the specific capacitance of the membrane is very high, close to 1 $\mu F/cm^2$. Having the properties of a capacitor, the membrane is able to separate electric charge, achieved by a *difference* in the number of anions and cations on each sideof the membrane; this charge separation, in turn produces a potential difference across the membrane. In a capacitor the potential difference V is related to the charge Q by

$$Q = CV \qquad (3)$$

where C is the capacitance. It is important to notice that in the case of the plasma membrane a small amount of charge separation is able to generate a large potential difference. For example, to obtain a membrane potential of 100 mV it is necessary to separate the product of $C_m = 1 (\mu F/cm^2)$ times $V_m = 0.1$ (Volts), that is, $Q = 0.1$ $\mu Coulombs/cm^2$. To get an idea of the magnitude of this charge, we can compute the number p of monovalent ions that must be separated across the membrane to explain this charge

$$p = 0.1 \times 10^{-6} \text{ (coul/sq cm)}/1.6 \times 10^{-19} \text{ (coul/ion)} =$$

$$6.25 \times 10^{11} \text{ (ion/cm}^2\text{)}$$

which corresponds to only 6250 ions per μm^2 of membrane.

The energy to put an ion into the lipid bilayer is so large that we would expect the membrane to be practically impermeable to ions. However, experimentally, it has been found that the membrane presents a finite permeability to cations and anions. Today we know that this permeability is mediated through specialized proteins that can act as carriers or channels for the passage of charged species. The detail of the operation of channels will be described later in this course. What is relevant here is the fact that ions can penetrate through specialized pathways which constitute the membrane electrical *conductance* (conductance is the reciprocal of resistance). This conductance will be another element of our electric circuit that will represent the electrical characteristics of the membrane.

Again we will define units that are independent of the membrane area. If we measure the electrical resistance of a membrane of 1 sq cm of surface area, it will be 10 times larger than the resistance of a membrane with the same characteristics but of 10 sq cm surface area. This is because the resistance decreases as the access area increases. For this reason, we can define the membrane specific resistance R_m in units of ohms sq cm or specific conductance G_m in units of Siemens/cm^2 (S/cm^2).

THE MEMBRANE POTENTIAL
The Nernst Potential

How is it possible to separate charge across the membrane? Let us take a simple example. Assume that we have a membrane separating two compartments (Fig. 1) that has channels that are only permeable to potassium and no other ions can permeate. Initially the channels are closed and we add 100 mM of KCl to the lower compartment (say the interior of the cell) and 10 mM of KCl to the upper compartment (the outside). As we have added a neutral salt, there will be the same number of cations than anions in the lower compartment and the same will be true for the upper compartment (even though the total number of ions is 10 times lower in the upper compartment).The consequence of the electroneutrality in each side will be zero charge difference across the membrane and consequently the membrane potential difference will be zero. This is because from eq. (3) we can write that $V=Q/C$, where V is the potential difference and Q is the excess charge. The permanent thermal motion of the ions will make them move randomly but they will not be able to cross the membrane because they are poorly permeant through the bilayer and the channels are closed. Suppose that at one point we open the channels. Then, as there are 10 times more K$^+$ ions in the bottom initial situation is schematically pictured in Fig. 1A where a K$^+$ ion (the black balls) is crossing the channel in the upward direction leaving a Cl$^-$ ion behind. This flow, which is proportional to the concentration gradient, increases the top compartment by one positive charge and the lower compartment by one negative charge, producing a charge separation (Fig. 1B). This charge separation introduces a non-random new electrostatic force acting on the ions, as compartment than in the top compartment, there will be 10 times more chances of an ion crossing up than down. This
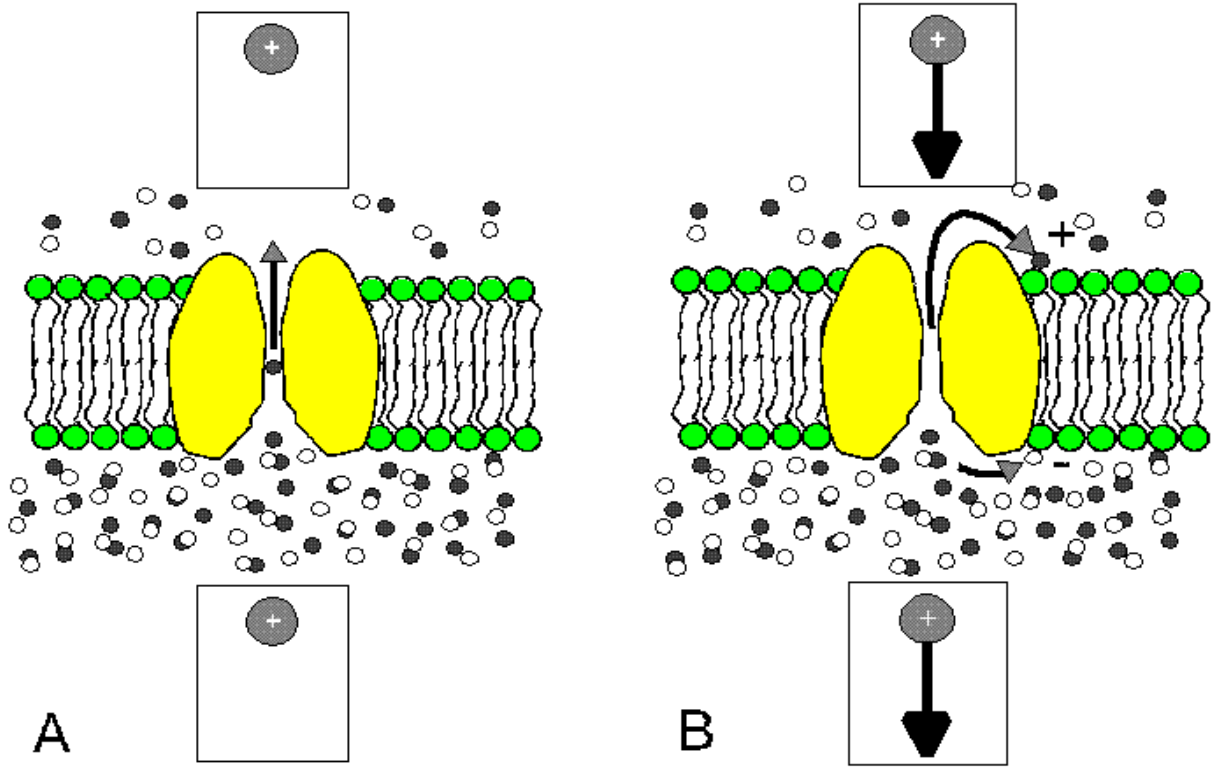
Figure 1. **A** membrane with a channel selective to cations (dark balls) separating a concentration gradient of a salt. **A.** After establishing the gradient, the channel opens and a cation goes from bottom to top compartment. **B.** After the first ion crosses the membrane, a potential is established as indicated by the + and - signs. **INSETS** show the electrical force acting on the ion in both conditions: none in A and the down arrow in B.

pictured in the box insets of Fig. 1. This electrostatic force tends to drive the ions from the top compartment into the bottom compartment and at the same time it tends to brake the flow in the opposite direction. The final result is that the charge separation will build up a voltage across the membrane ($V=Q/C$) that will continue to increase until the flow in both directions becomes equal due to the increased electrostatic force that will tend to balance the flow produced by the concentration gradient. When that happens, any ion that crosses in one direction will be counterbalanced by another crossing in the opposite direction, maintaining an equilibrium situation. This potential difference is then called the *equilibrium potential* or Nernst potential.

The above discussion can be put in more quantitative terms by expressing the net ion flow j in terms of the chemical and electrical gradients:

$$j = -D\left[\frac{dC}{dx} + C\frac{zF}{RT}\frac{dV}{dx}\right] \tag{4}$$

where D is the diffusion coefficient, C is the concentration, R is the gas constant, V is the voltage, z is the valence, F is the Faraday constant, and T is the temperature. When $j=0$ (no net flow), eq (4) can be integrated and we get:

$$V = \frac{RT}{zF}\ln\frac{C_o}{C_i} \tag{5}$$

which is the Nernst equation that relates the voltage V across the membrane which is in equilibrium with the concentration gradient established by the concentrations C_o, outside, and C_i, inside. It is customary to call this voltage4 the equilibrium potential of the ion "N" E_e, and by calling the external and internal concentrations of the ion "N" N_o and N_i, respectively we can rewrite eq. (5) as follows:

$$E_N = \frac{RT}{zF}\ln\frac{N_o}{N_i}$$

More than one specific channel.

In the nerve membrane there are several types of channels, each of which is selective to an specific ion, such as Na^+ or K^+. Therefore the situation of zero net flow across the membrane does not depend on one particular ion concentration gradient but it involves the concentration of the other permeant ions and their relative permeabilities. In this situation we have to consider the individual fluxes j_{Na}, j_K, etc. and the solution when the sum of all the flows is zero gives the Goldman-Hodgkin-Katz equation which can be written as

$$E = \frac{RT}{F}\ln\frac{P_{Na}Na_o + P_K K_o + P_{Cl}Cl_i}{P_{Na}Na_i + P_K K_i + P_{Cl}Cl_o} \tag{7}$$

where the permeabilities for the ion k is written as Pk and the concentrations of the ion are given by its chemical symbol followed by the subindex indicating the side of the membrane, with i for inside and o for outside. Thus, according to this equation, the voltage across the membrane is determined by the concentrations of all the ions and is most affected by the ion with the highest permeability. If E, as computed from eq. (7) is equal to the E of the Nernst equation (eq. 6) for one particular ion, we say that that ion **is in equilibrium**.

Real channels are not perfectly selective. The selectivity of ion channels is not perfect and, for example in K^+ channels, for every 20 K^+ ions that flow through the channel, one Na^+ ion can get through. This means that we cannot apply the Nernst equation to compute the potential that produces zero flow across the channel because more than one ion is involved. Instead, we could use the Goldman-Hodgkin-Katz equation (with $P_K/P_{Na}=20$, in the case of the K channel) and the potential predicted by the equation would be called *reversal potential*, instead of equilibrium potential, at which the net flow of charge through the channel is zero. .

> The program MEMB simulates the fluxes and potentials using random motion for the charged species
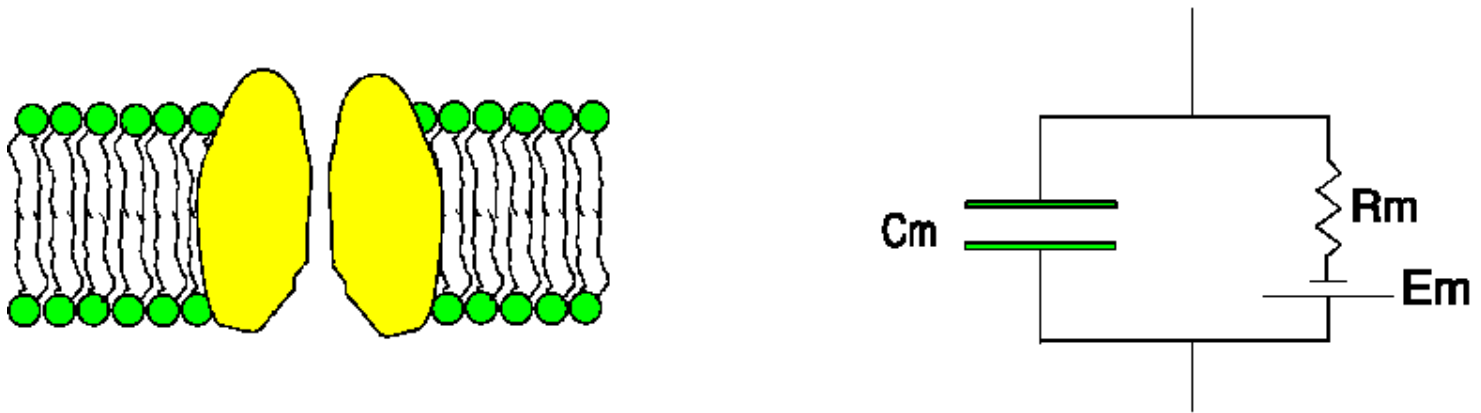
THE ELECTRICAL MODEL OF THE MEMBRANE

Figure 2. The origin of the electrical equivalent circuit of the membrane

We have now described the capacitance of the membrane, mainly given by the bilayer, the resistance of the membrane given by the ionic channels, and we must now include the *membrane potential*. As explained in the previous paragraphs, this membrane potential exists even in the absence of stimulus or external electric field due to the charge separation produced by the ion redistribution under the influence of chemical and electrical gradients. In resting conditions, this voltage is called the *resting potential* and it can be represented as a battery that must be in serieswith the membrane resistance (Fig. 2). This battery of electromotive force [3]E_m and the membrane resistance R_m may be considered as the equivalent electric circuit of the membrane of the axon, which includes all the membrane resistances and batteries of the different systems of ionic channels each one with its own conductance g_i (where $g_i = 1/R_i$) and reversal potential E_i. Notice that the battery is in *series* with the resistance which implies that the membrane potential is equal to E_m only when there is no current flow through the resistance R_m, that is, in open circuit conditions, or when it is measured electrometrically (without draining current through the membrane).

> ## CONVENTIONS
> **Membrane potential (V)** is measured as the difference in potential between the inside minus the outside ($V_{inside} - V_{outside}$).
> **Membrane Current (I)** is positive when positive ions (cations) flow from the inside to the outside of the cell. For cations, **outward current is positive** and **inward current is negative**. For anions, an inward (negative) current corresponds to an outward flow of anions.
> In all diagrams, the **outside of the cell is up** and the inside of the cell faces down.

The circuit in Fig. 2 is a minimal representation of the membrane. It is important to note that this representation refers to a membrane element of very small dimensions, such that the potential could be considered constant along each side of the membrane. This is only true if the element is made infinitesimally small bacause any finite size may not be isopotential due to the electrical resistance of the medium and the geometry of the axon. As we will see below, in the case of the axon we will have to locate this basic circuit of the membrane element in the cylindrical geometry of the axon which will make the final circuit quite complicated. But before incorporating the geometry, we can study the basic properties of our elemental circuit which will be extremely useful in understanding the electrical behavior of the elementary unit that constitutes the axonal membrane.

Summary:

Measurements are done electrically, therefore electrical equivalents are useful to understand membrane function:

The lipid bilayer = = capacitance
The channels = = conductances
The ionic gradients = = batteries

Basic Capacitance properties:

$$Q = C V$$

the charge Q is proportional to the voltage V across the capacitor and the proportionality constant is the capacitance C.

Separation of charge: To produce 100 mV across a C of 1 uF/cm², one needs 10^{-7} coulombs/cm² or 10^{-15} coul/um². 1 mol of monovalent ions is 96500 coulombs (F = 96500 coul/equiv) and 1 mol is 6×10^{23} ions (Avogadro's number $L = 6 \times 10^{23}$ mole⁻¹), therefore 6000 ions/μm² are required to produce a $V = 100$ mV.

The current needed to charge the capacitor is

$$I = dQ/dt = CdV/dt$$

Note that the current is proportional to the *rate of change* of the voltage. When the voltage is constant there is NO capacitive current. More on this, later.

Basic current equation through the conductive pathways:

The current through the conductive part of the membrane can be expressed as a product of a conductance and a driving force:

$$I = g(V - E_e) \tag{8}$$

where $V - E_e$ is the driving force and g is the conductance (reciprocal value of resistance, $R = 1/g$) expressed in Siemens(S = 1/ohm). V is the membrane potential and E_e is the reversal potential for that pathway. If the pathway is selective to only one ion species, E_e corresponds to the equilibrium potential of that species e (the potential predicted by the Nernst equation).

There are several types of conductance and their classification is done according to the type of channel involved. Thus, we have: Sodium, selective to Na; Potassium, selective to K; Chloride, selective to Cl; Calcium, selective to Ca, etc. Currents through each conductance type: I_{Na}, I_K, I_{Cl}, etc

The Resting Membrane Conductance

In the **resting membrane**, the resting potential V is constant and there is *no net current* crossing in or out of the cell, although each individual conductance may be carrying a net current, therefore:

$$I_{Na} + I_K + I_{Cl} = 0 \tag{9}$$

Replacing the current expression for each conductance, from eq (8) into eq (9):

$$g_{Na}(V - E_{Na}) + g_K(V - E_K) + g_{Cl}(V - E_{Cl}) = 0 \tag{10}$$

Solving for V in eq (10) we get:

$$V = \frac{E_{Na}g_{Na} + E_K g_K + E_{Cl}g_{Cl}}{g_{Na} + g_K + g_{Cl}}$$

<div align="right">(11)</div>

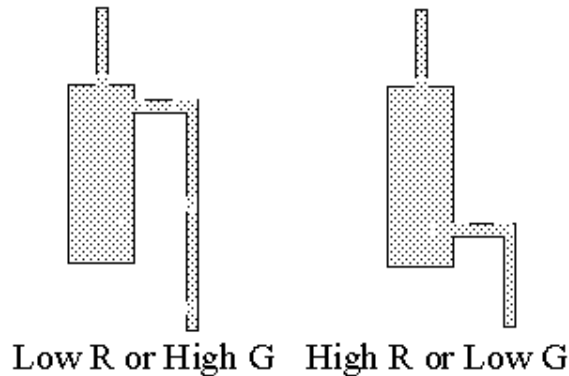Low R or High G   High R or Low G

Figure 3. Representation of the variable ionic conductance. The cursor can slide up and down to represent a change in the total value of the resistance. **NOTE**: the maximum value of the conductance may differ among the different variable resistors in a circuit.

This expression is similar to the Goldman-Hodgkin-Katz equation but it considers conductances and reversal potentials instead of permeabilities and concentrations. In several excitable membranes, such as the squid axon, g_{Cl} is small but there are other type of conductances that have been lumped together under the name of leakage or g_L, which is then substituted for g_{Cl}.

Simulation of the membrane potential using the Equivalent Circuit Program.

When simulating the membrane potential using this link, move the slider with the mouse to modify the conductances. In this section, we will study the currents through the different ionic pathways and the resulting voltage across the membrane. The conventions will be that the inside of the membrane faces down and all the voltages will be referred to the outside. For example, if the membrane potential is -80 mV it means that the inside is negative with respect to the outside. The variable conductances (or variable resistances) are represented by a box with a side cursor (or arm) that can slide up and down the side of the box (see Fig. 3). Thus, when the cursor is close to the top the resistance between the two ends is minimum and becomes maximum when the arm is close to the bottom (Fig. 3). The equilibrium (or reversal) potentials are represented by batteries with the value written near the battery symbol. In addition, the intensity of the current is represented by the degree of shading of the wires, being black the highest intensity and white zero current. The arrows indicate the direction of positive ions current flow. With these conventions, we can study the influence of the conductances and reversal potentials in the final membrane potential in the steady state.

First Case. Only one conductance is present (Figs 4A and 4B). All the other conductances are zero (that is, resistances are infinite) represented by an interruption in the connection of the variable resistor. This means that there is no current circulation in any of the branches with infinite resistance. This is represented in Fig. 4A for the case of finite K conductance and all the others zero. Notice that the membrane potential will be equal to the K equilibrium potential (in this case -73.3 mV) because there is no current circulation through any other resistor and, since we are considering steady state, the capacitor does not drain any current (we will study later the origin of capacitive current).
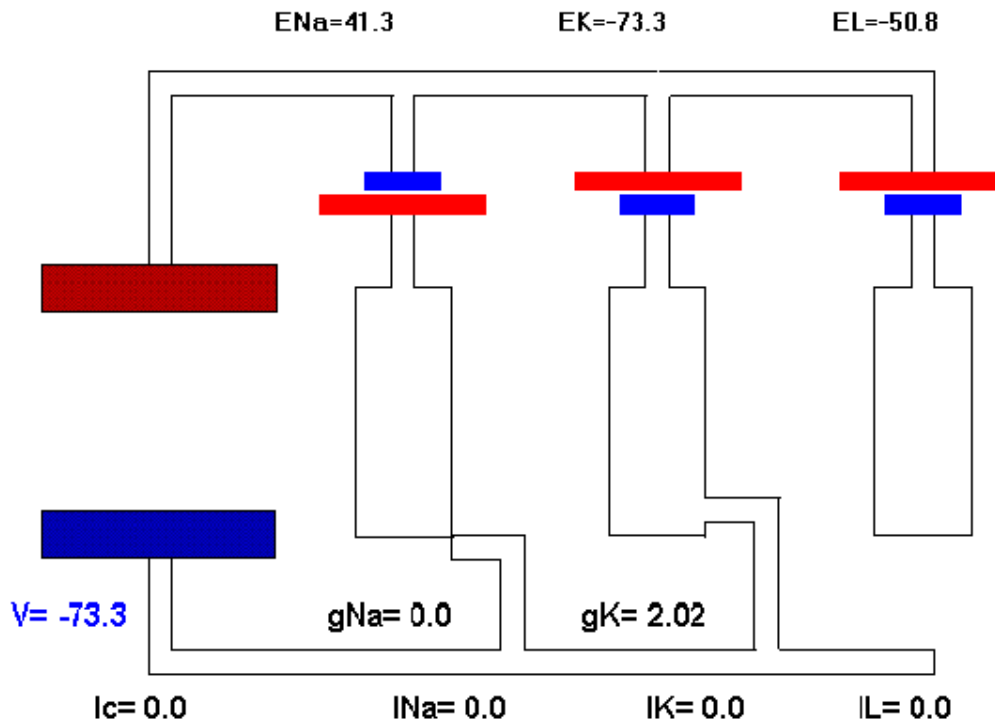
Figure 4A. Equivalent circuit with only g_K gives a resting potential equal to E_K.
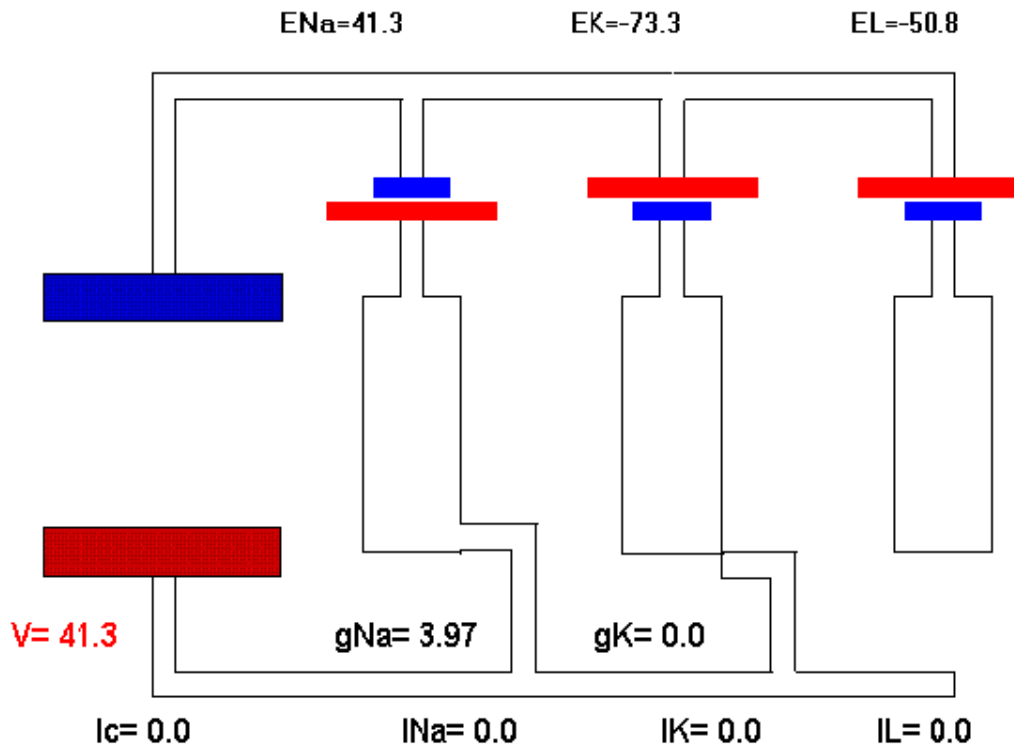

Figure 4B. Equivalent circuit with only g_{Na} gives a resting potential equal to E_{Na}.

In Figure 4B there is a representation of the case when only the Na conductance is present and all the others are zero. As expected, in this case the membrane potential will be identical to the Na reversal potential, in this case +41.3 mV. The situation depicted in Fig. 4 is a rough approximation of what occurs during an action potential: at resting the membrane is selective to K and the membrane potential is near -73 mV and during the action potential the membrane becomes selective to Na and the membrane potential reverses and becomes positive.

ENa=41.3   EK=-73.3   EL=-50.8

V= -60.8   gNa= 0.02   gK= 0.37

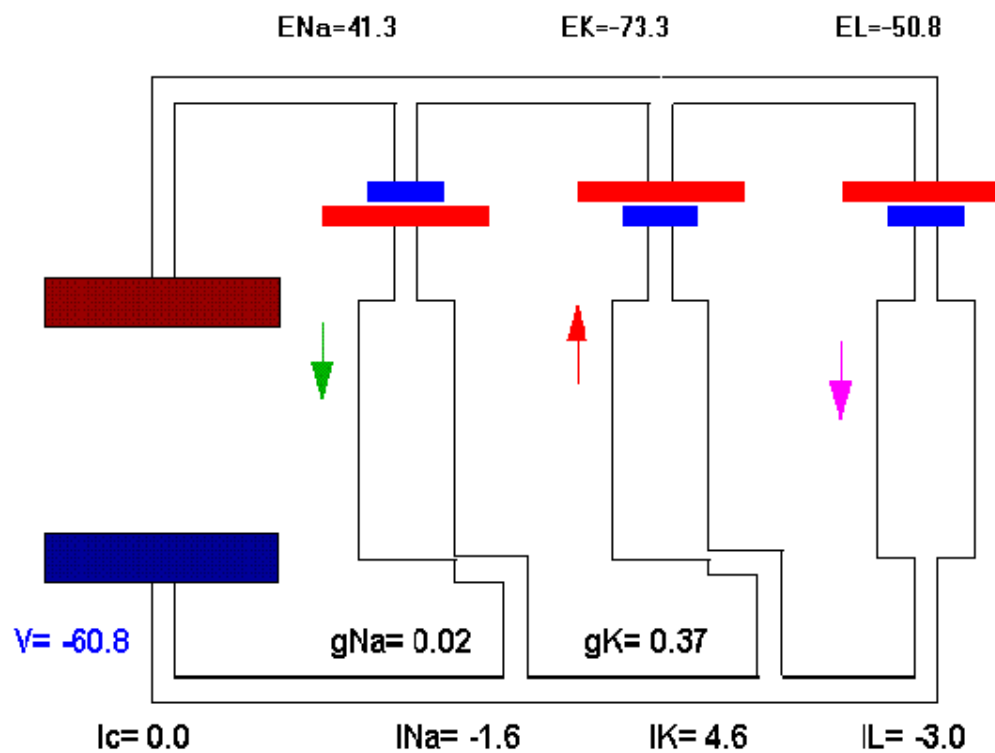Ic= 0.0   INa= -1.6   IK= 4.6   IL= -3.0

Figure 5. The equivalent circuit of the membrane of the axon in the resting state. Both Na and K conductances are very small (cursors are close to the bottom of the variable resistors) but the K conductance predominates (0.37 against 0.02) and the membrane potential is close to the value of E_K.

ENa=41.3   EK=-73.3   EL=-50.8

V= 31.4   gNa= 29.50   gK= 2.55

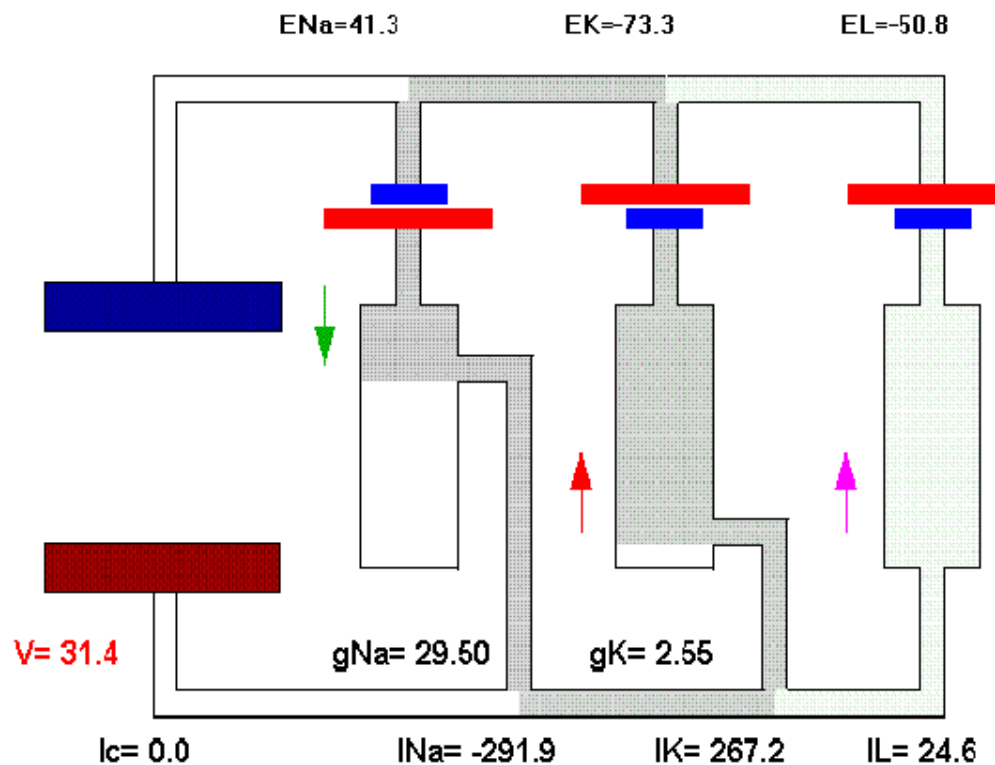Ic= 0.0   INa= -291.9   IK= 267.2   IL= 24.6

Figure 6. The equivalent circuit of the membrane during the peak of the action potential. The Na conductance predominates, therefore the membrane potential is close to the value of E_{Na}

Second Case (Figs. 5 and 6). Now we consider the more realistic situation where all the conductances have a finite value, although different among them. In the resting condition, the predominant conductance is potassium as shown in Fig. 5. In the resting condition all the conductances are small but the K conductance is larger than all the others. Notice that the relative values of the conductances (in mS/sq cm) are $g_K=0.37$, $g_{Na}=0.02$ and $g_L=0.3$. The K battery will drive an outward current through the K conductance that will be drained by the leakage and Na branches to make the total current equal zero. The resulting membrane potential will be between the K reversal potential and Na reversal potential, but closer to the E_K because g_K is the largest conductance. Finally, let us consider the case when the Na conductance predominates as during the rising phase of the action potential. In this case, the actual sodium conductance increases several fold over the resting condition, becoming the dominant conductance. This produces a large flow of inward current through the Na conductance that is drained out by the K and leakage conductances (see Fig. 6).

The *action potential is the result of changing the membrane from a K selective condition to a Na selective condition*. We must explain now how this occurs. To understand how these conductances shifts occur, we must study their ability to change with the membrane voltage, or their "voltage dependence".This leads to a description of voltage dependent conductances. In voltage dependent conductances the value of the conductance depends on the membrane potential.

NOTES:

1. A **potential difference** exists between two points whenever the introduction of a conducting path between the two points would result in spontaneous charge transfer or electric **current**.

2. An electric **capacitor** is a device that allows the separation of electric charge on two conducting plates by a material, or dielectric, that is not able to sustain an electric current.

3. The electromotive force of a battery is the voltage measured from the battery in the absence of current circulation.

VOLTAGE DEPENDENT CHANNELS

Channels are specialized membrane proteins that let ions pass through at a high rate when they are open. They are thought to have a specialized pathway that allows the conduction of ions. This pathway may be open or closed depending on the conformation of the channel molecule. Thermal motion will cycle the protein between the closed and open conformations; consequently this transition is a random event. This means that it is not possible to predict at any given time whether the channel will be open or closed. The laws of probability, however, allow us to make certain predictions of the average behaviour of the channel. Thus, by observing the channel operation for a long period of time, we can compute the total time the channel is open (O) as the sum of all the individual openings ($O = O_1+O_2+...$) and the total time the channel is closed (C) as the sum of the individual closed times ($C = C_1+C_2+...$). We can estimate the probability of being open, P_o, as

$$P_o= O/(O+C)$$

which is equivalent to the fractional open time.

In the type of channels that we are particularly interested, the P_o is dependent on the membrane potential, thus they are called voltage-dependent channels. Na and K channels in neurons are voltage dependent and their open probability is
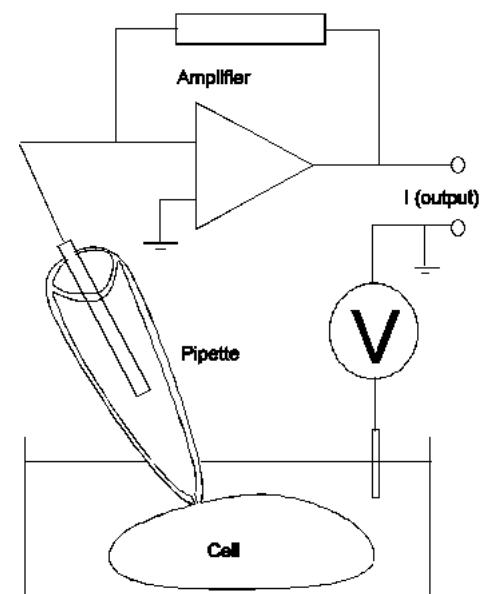

Figure 7. The patch clamp technique allows the recording of the current through one channel.

low at negative (hyperpolarized) potentials and is high at positive (depolarized) potentials. The summation of a large population of these voltage dependent channels will produce a voltage dependent conductance in the cell membrane. Our approach will first be to understand the operation of the channels and then to explain the interaction between current and voltage across the axon.

Single channel recording.

The current through an open channel is very small, of the order of 10^{-12} Amps or pA. This is not surprising because it is the result of only one molecule allowing the ions to go through. In fact, we may consider the ion channel like an special "transducer" that allows us to detect its activity by recording the single channel current. The patch clamp technique (Fig. 7) is used to perform the single channel recordings and it is based on the isolation of a small area of membrane with a glass pipette with a small aperture (about 1 μm diameter). This pipette is sealed against the plasma membrane and when there is only one channel in the area encompassed by the pipette, the current through that channel can be recorded by a low noise amplifier (Fig. 7) while the membrane potential (V in Fig. 7) is maintained constant. This technique allows us to measure the flow of ions through one channel and calculate the actual open times O and closed times C.

The voltage-dependent potassium channel.

Several K channel genes have been cloned and sequenced, allowing the deduction of the amino acid sequence of the protein. There are six membrane spanning regions (see Fig. 8) and those regions have been identified with specific functions of the channel. The actual channel is made of four subunits which gives some symmetry to the molecule (see Fig. 8). Within the sequence of these K channels, membrane spanning segment 4 (S4) contains a peculiar sequence of 5 to 8 positively charged amino acids that are repeated every three residues. There is experimental evidence that this region is involved in sensing the membrane voltage and therefore controlling the open probability of the channel. In the series of figures of the Channel Operation of the web site (Plate 1) a simplified and idealized view of the channel is presented whereby the S4 segments are shown as cylinders that can move within the protein. The voltage across the membrane will have an influence on this movement because this segment is positively charged and we can speculate a possible mechanism for the control of P_o. We assume that when ANY of the four S4 segments are displaced toward the inside (only 2 positive charges shown in each S4 segment) the pathway for ion conduction is blocked. The only way to make it conductive is to move out and rotate ALL four S4 segments. In Plate 1, part E, this is shown for a depolarized potential (+20 mV) allowing the ions to permeate. The actual permeation pathway is lined by segment S6 and the loop between S5 and S6 and is represented by the green cylinders which are coupled to the S4 segment trough a linker, so that when the S4 is tilted up and straight , the corresponding region in the pore opens up. However, conduction will only occur when ALL four open up.

The movement of the gating subunit (the charged cylinder or S4 segment) is a *random event* and will occur extremely fast and spontaneously as indicated by the spikes (Gate-1 through Gate-4) in Fig. 9, (where an upward spike is a transition from resting to active and a downward spike is the opposite).
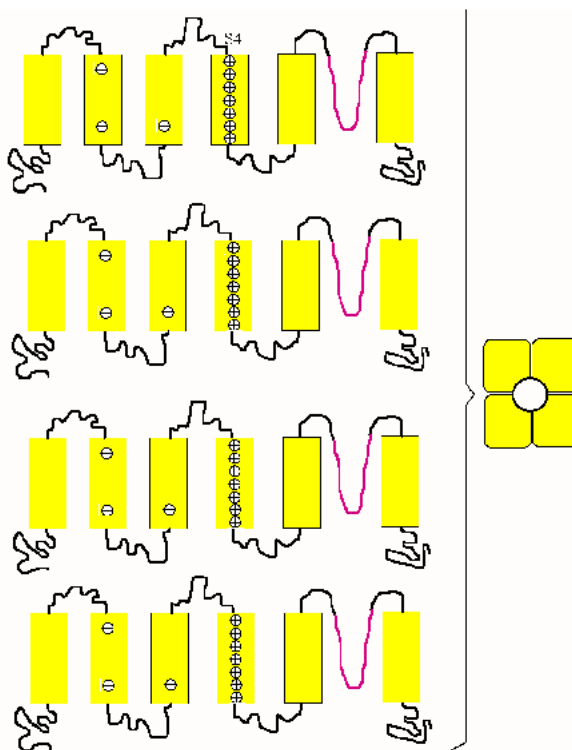

Figure 8. Each subunit of the K channel (left) has 6 transmembrane segments (S1 through S6). The S4 segment contain several basic residues. Between segment 5 and 6 there is a loop that, along with the S6 segment lines the conduction pore. Four of these subunits are assembled in one functional K channel (right)

However, the voltage across the membrane will have an influence on the probability that the segment will be straight down (resting) or tilted out (active). Thus, at negative potentials (the resting potential) the S4 segment will spend most
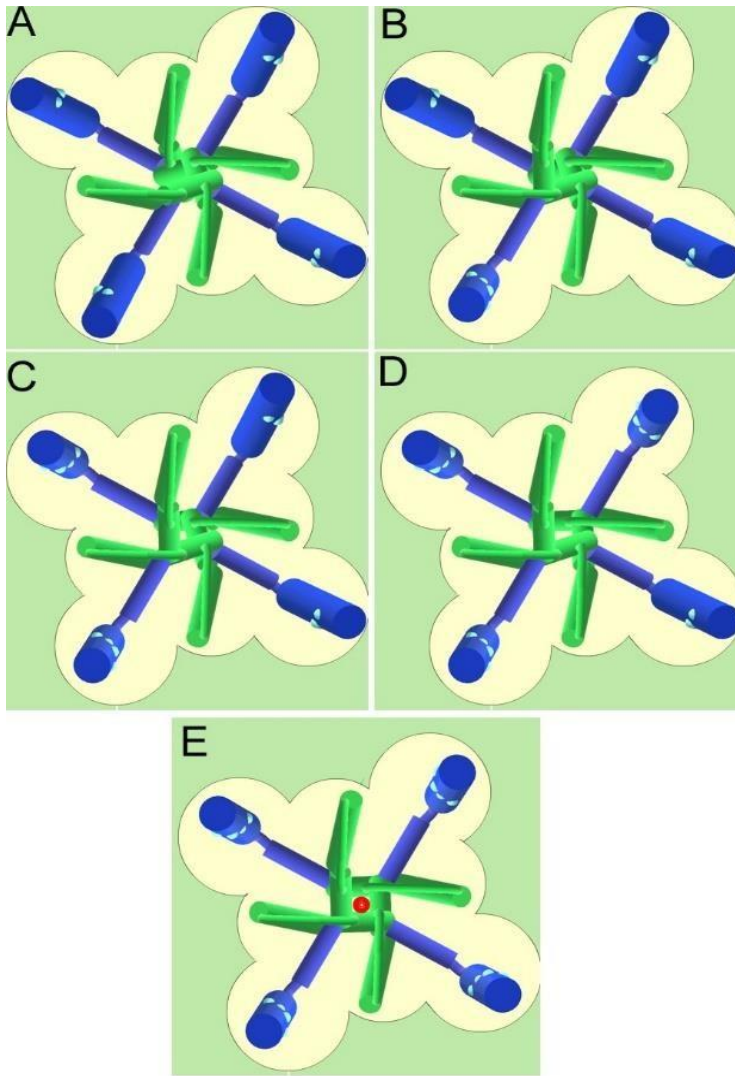

Plate 1. Schematic representation of the closed and open states of the K channel. View of the channel from the extracellular side.The pore lining is represented by the green segments and the S4 segments are pictured in blue. The coupling between the S4 segment and the pore is represented by the blue linker. **A.** view of the four subunits, all in the resting position. **B**. One subunit in the active position. **C**. Two subunits in the active position. **D**. Three subunits in the active position. **E.** The four subunits in the active position which makes the channel conducting (the K ion permeating is indicated in red). Notice that there are 16 possible conformations (2 x 2 x 2 x 2) but only one is conducting (E).

of the time in the resting position as it is attracted toward the inside of the membrane. On the other hand, at positive potentials, the S4 will tend to sit more in the active position due to electrostatic repulsion. In this way, the membrane potential is controlling the open probability because conduction will occur *when all four* are in the active position. If we call *n* the probability that any of the four cylinders is in the active position, then the probability that ALL four are in the active position is *n raised to the fourth power*. This means that the probability of being open will be

$$Po=n4$$

Starting from a hyperpolarized potential (-100 mV), most of the time the subunits will be in the resting position and very infrequently all four may be, for a brief period of time, in the active position producing a brief ionic current through the channel.

When the membrane potential is suddenly made more positive, there will be a period of time (a lag) before we may find all four in the active position, producing an initial delay in the first opening of the channel (trace labeled iK in Fig 9). Even if the potential is maintained positive, it is quite likely that one of the subunits will make a sojourn to the resting position producing a brief interruption in the single channel current (Fig 9).
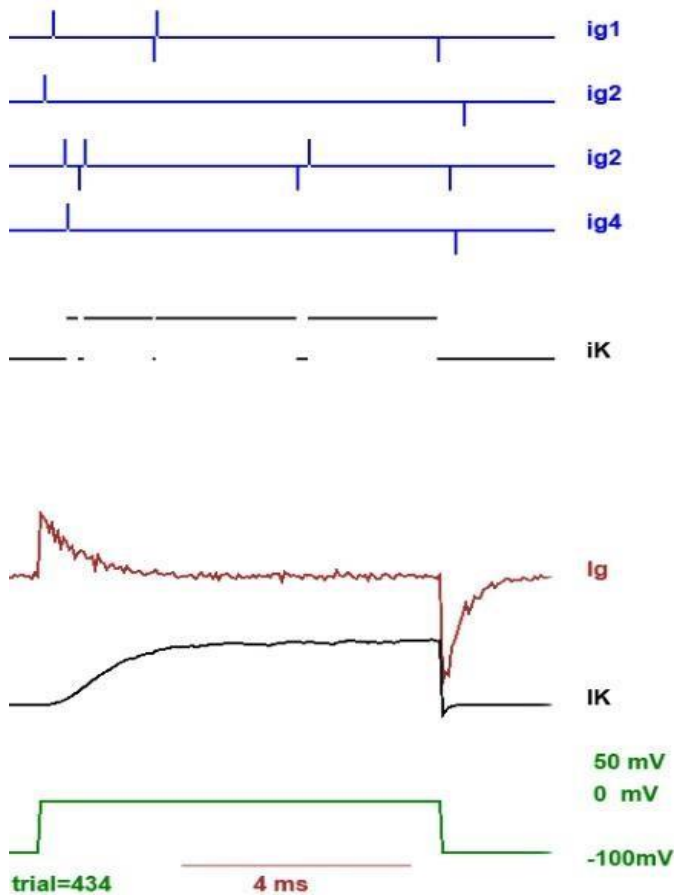
Figure 9. Electrical manifestation of the movement of the gating charges. All traces are in response to the pulse pattern indicated in the bottom grenn trace (start at -100 mV pulse to zero and returning back to -100 mV). The first four blue traces (ig1 through ig4) show the gating charge shots of each of the four voltage sensors: upward spike is transition to active and a spike down is transition to the resting state. The next trace, iK, (black) shows the ionic current through the channel. The next trace (brown) is the average after 434 trials (in this case) of all the gating charge transitions, making up the macroscopic gating current (Ig). The next trace (black) corresponds to the average of the single channel ionic currents after 434 trials making up the macroscopic ionic current (IK).

The vertical spikes in the traces Gate 1 through Gate 4 in Fig. 9 represent the rapid transfer of charge of the gate itself across the membrane (currents shots) which is called the single gate gating current. When many of these traces are averaged, these shots of current add up into what is called **Gating current** (avg Ig in Fig. 9) because it is an electrical manifestation of the movement of the voltage sensor. These current shots *should not be confused* with the single channel current: shots are very brief, transient currents that represent the movement of the voltage sensor while single channel currents are the expression of the movement of ions through the open channel.

As the depolarization is increased, the latency to first opening decreases and the open times are prolonged (see Figs 10, 11 and 12). These are *kinetic* features of the single channel currents and they are important in determining the characteristics of the macroscopic currents. In fig. 10, the membrane potential was taken to -30 mV and very few openings occurred (the recording of the single channel current for only one trial is shown, in addition to the average of 807 trials). The average current (IK) reflects the contribution of 807 trials to the same potential or, as the channels are assumed to be independent, it is equivalent to the current produced by 807 channels in response to the voltage step to 30 mV. Notice that as the potential is made more positive (Figs 11 and 12) the average current increases and reaches its final value in a shorter time. This is a consequence of a combination of i) the higher open probability at more depolarized potentials, ii) an increase in the driving force ($V-E_K$ is larger) and iii) the decrease in the first latency time.
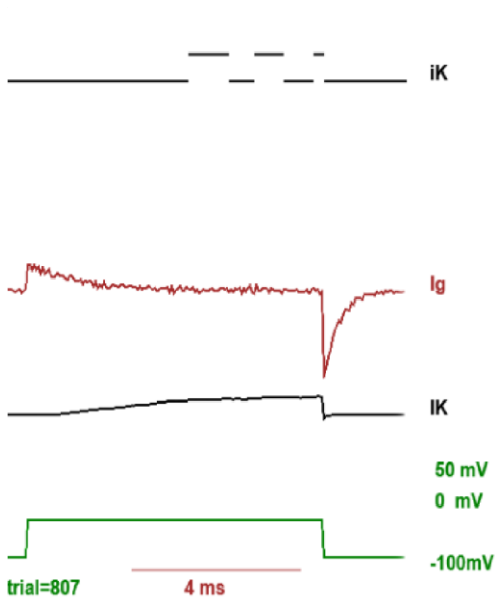
14

Figure 10. Pulse to -30 mV. Notice that the channel opens infrequently and the long latency of opening.
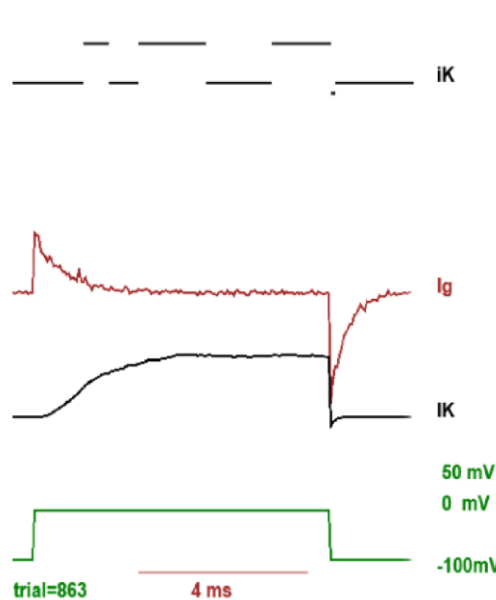
Figure 11. Pulse to -10 mV. Latency is decreased and open probability is increased. Also, single channel current is larger because the driving force is increased
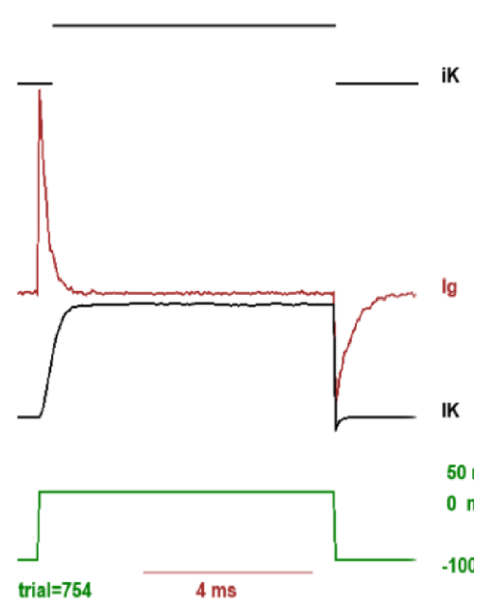
Figure 12. Pulse to 20 mV. Notice that the open probability is close to 1 and the latency to first opening is very short.

Single channel conductance. There are at least two important measurements that we can make in the channel behaviour as a function of membrane potential. The first one is the current that flows through one channel as a function of membrane potential. This can be done by pulsing the membrane at different voltages and measuring the amplitude of the currents when the channel opens as shown in the examples of Figs. 10-12. The relation between the single channel current and membrane voltage is very close to a straight line that crosses the horizontal axis at -80 mV, the K equilibrium potential. This means that the channel, when it is open, behaves like an ohmic resistor and the single channel current is given by

$$iopen\ single = g(V - EK)$$

where g is the single channel conductance and E_K is the equilibrium potential for K. (see top panel of Fig. 13). (This is only an approximation because depends on the K concentrations on both sides and V)

Open probability. The second important measurement is to compute the P_o as a function of membrane potential V. By inspection of Figs 10 to 12, it is clear that P_o not only depends on V but also on time because at short times after giving the depolarizing pulse no openings are observed but later they appear more frequently. At long times, however, this P_o is stabilized and its functional dependence on V is sigmoidal, as shown in the middle panel of Fig 13. This is the result of the voltage influence on the position of the gating subunit which controls the opening of the channel.
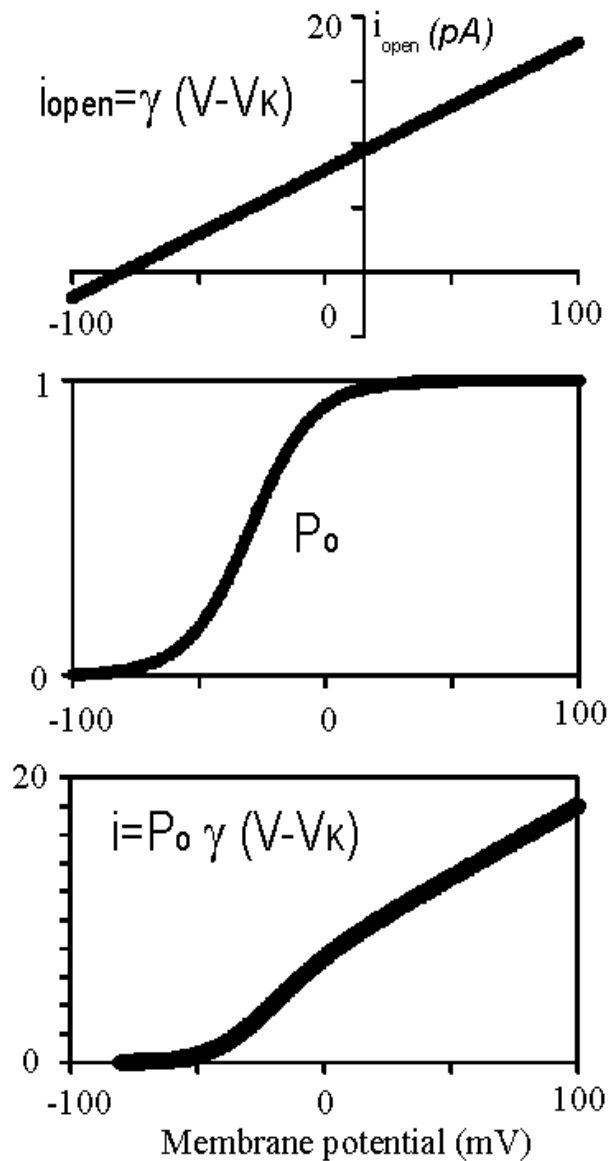
15

Figure 13. Top: the current through an open channel i, as a function of V. Middle: the open probability as a function of V for a long pulse (steady state). Bottom: the actual current.

Summary
- The K channel has only two levels of conductance: open or closed.
- When open, the channel follows Ohms law: $i = \gamma(V - E_K)$
- The probability of being open increases with depolarization
- The time it takes to reach the new probability when depolarized is shorter with larger V.

Current as a function of voltage. To compute the current at any instant, we need to know the probability of being open and the current through an open single channel. The current will be given by

$$i = P_o g\ (V - E_K).$$

(see bottom panel of Fig 13). This current i will be in general a function of time because P_o is a function of time.

Macroscopic K currents. The time course of a single K channel opening is not predictable but the average behavior is easily obtained by summing a large number of single channel events in response to the same voltage perturbation. This

is shown in the lower traces labelled IK of Figs 10 to 12. The long latency for small depolarizations is translated in a slow rise of the average current (Fig 10), while a short latency for large depolarizations is shown as a very fast rise in the average current (Fig. 12). For example, in the squid axon there are about 50 to 100 K channels/μm^2 which means that a small region of the axon will have a large number of channels contributing to the total current. If these channels operate independently from each other, then the average behaviour obtained by repeating the same stimulus in one channel will correspond to the behaviour of the large population of channels in the cell. This is an important result because we can predict the macroscopic current by repeating the same observation in one channel. We can compute the macroscopic current density as the single channel current times the density of channels N_K and it will be given by

$$I_K = N_K \ g \ P_o(V-E_K)$$

which is proportional to the current i shown in the bottom panel of Fig. 13. Notice that the macroscopic current $I_K = i \ N_K$ is a non-linear function of V but that most of the nonlinearity is produced by the nonlinear characteristic of the P_o vs V and not by the i-V characteristics of the open channel.

The Voltage dependent Sodium Channel

Many Na channels have more than one subunit of different size and structure. However, most of the properties we will discuss reside in the subunit which is about four times larger than the individual subunit of the K channels. It has four homologous domains, each containing 6 putative membrane spanning segments (see Fig. 14). Therefore the structure of the molecule is considered to be similar to four K subunits. From the functional point of view there are important differences between Na and K channels. First, the Na channel is mainly selective to Na, allowing K to pass about 15 times less easily. In contrast, K channels are more selective to K, allowing Na to pass with a permeability less than 20 that of K. Another striking difference is the speed of activation. The latency to first opening is about 10 times shorter in Na channels than in K channels, giving Na channels an overall activation time about 10 times faster than K channels. Another important difference is the presence of inactivation (see below) which does not occur in the delayed rectifier K channel previously described.
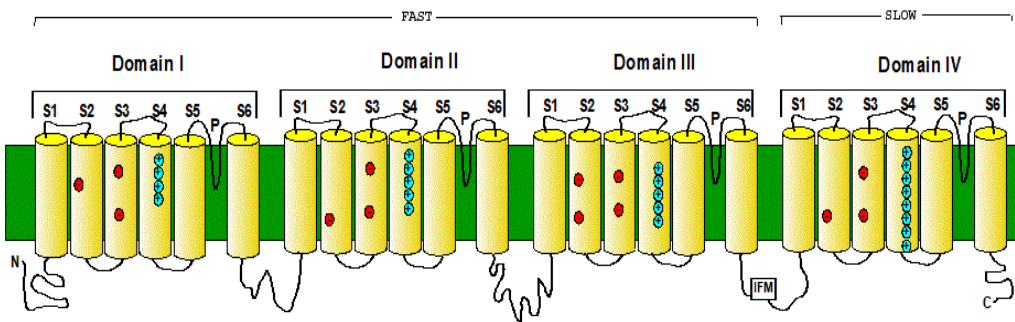

Figure 14. The alpha subunit (pore forming subunit) of the Na channel containing four homologous domains. The first three domain have fast kinetics while the 4th domain has slower kinetics. The IFM (isoleucine, pchannel. henylalanine, methionine) motif is reponsible for the inactivation of the

In the Na channel we can distinguish three main states of the channel: **closed**, **open** and **inactivated**. As it is the case of the K channel, the opening of the Na channel also requires several gating events (in the classical formulation: three events) and proceeds by a similar mechanism as explained above for the K channel. Plate 2 shows a simplified view of the Na channel: pore lining is shown in green (selectivity filter and S6 segments) and the movement of the voltage sensors are indicated as a displacement of a charged cylinder (S4 of domains I through IV), affected by the membrane potential. If the S4 segment of Domains I, II and III are simultaneously up , the channel opens. However, once the channel is open, even if the depolarization is maintained, conduction stops (see Plate 2). This corresponds to the onset of inactivation. The inactivation occurs as following: the S4 segment of domain IV (red) responds to a voltage change with slower kinetics than the S4 segments of domains I-III. But when S4 of domain IV moves up, the IFM motif docks and binds into its site resulting in a conformational change on the S6 segments (green cylinders) of domain III and IV

that exposes four large hydrophobic residues into the pore, thus blocking conduction (the inactivated state). In this condition, the channel is *still open but unable to conduct.* In the macroscopic current, the IFM motif binding and the subsequent block by the 4 hydrophobic side chains produces a decrease of the ionic current during the maintained depolarization. Important features of the inactivation mechanism are its establishment and removal.
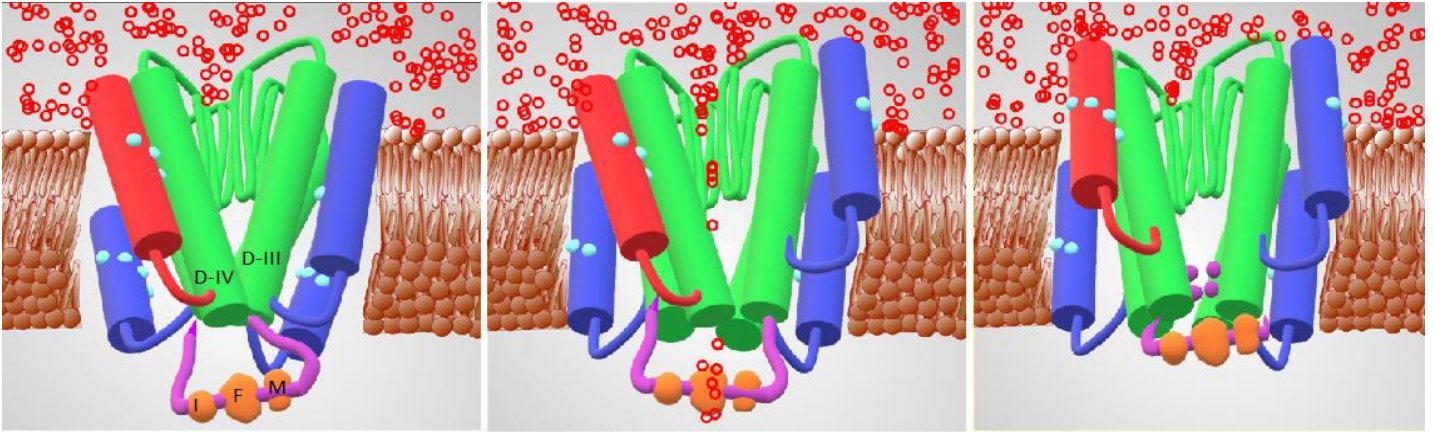

Plate 2. Schematic representation of the Na channel operation with sensors of Domains I, II and III in blue (fast) and sensor of Domain IV in red (slow). All four sensors couple to the bottom of S6 segments (in green) to control the gate. The channel conducts when Domains I,II and III are *simultaneously* active (m^3, according to the Hodgkin and Huxley formulation) but it also conducts when all four Domains are active. However when the sensor of Domain IV becomes active, the IFM motif gets in position and the side chains of four bulky residues in S6 segments of domains III and IV blocks conduction.
LEFT: Closed State. This state is favored at hyperpolarized potentials (in this case just before the pulse, -100 mV). This is one of the many possible closed and non-inactivated states.
OPEN: Open State (conducting). This state is favored by the depolarizing pulse to -10 mV. Ions cross the pore and go from tthe external solution to the internal solution.
RIGHT: Inactivated State. Even though the membrane is still depolarized and the gates are in the open position, the channel is not conducting because the pore is blocked by the 4 bulky amino acids (in violet) that are moved in position after the IFM motif binds.

It is clear from Fig. 15 that the probability of opening increases with depolarization as was the case of the K channel. The single channel event is larger at hyperpolarized potentials (compare -50 with 20 mV) because there is a larger driving force. At +60 mV the single channel current is outward because that potential is more positive than +40 (the equilibrium potential of Na for this ionic condition). Although the single channel event is large at -50 mV, the average current (top trace) is almost negligible because the events occur rarely at this potential, that is, the P_o is very small at 50 mV. On the other hand, at +20 mV the open channel currents, although smaller, are much longer because the P_o is higher. Following the same ideas presented for the activation of the K channel, we can express the P_o of the Na channel to be proportional to m^3, where m is the probability that any of the three gating subunits are in the active position. To consider the effect of the blocking ball we may define the probability that the ball is *in the blocking position* as *1-h*, or *out of the docking site* as *h*. In that case, the open probability is given by

$$P_o=m^3h$$

if the activation of the gating subunits and the ball docking were independent events. As it is seen in Plate 2 and in the Na simulation, the IFM motif will get in its site after the channel is open and much less frequently it will dock in the site when the channel is closed. This means that the inactivation process is *coupled* to the opening (or activation) of the channel, that is, the two events are not independent.[4] The consequence is that the voltage dependence of the inactivation process is practically all derived from the voltage dependence of activation. This means that the inactivation IFM motif does not need a voltage sensor of its own. We know now that when the sensor of Domain IV goes into the active position then the probability of the inactivating IFM particle to get into position increases drastically such that it is a good approximation to say that the inactivating particle blocks conduction *only* after Domain IV sensor is active. As Domain IV kinetics is slower than Domains I, II and III, the binding site exposed by

Domain IV movement is only available after the channel is open and the voltage dependence of inactivation is inherited mainly from the voltage dependence of Domain IV.

What happens when the depolarization is terminated and the membrane is returned to negative values? The channel deactivates but it cannot do it completely until the IFM motif exits from its binding site and this happens very slowly. For example, returning to -70 mV it takes an average of more than 10 ms for the IFM to exit. This has an important consequence because if one depolarizes the membrane again after a period of only 2 or 3 ms at -70 mV there will be no channels able to conduct because the IFM is still in position, hence the channel is inactivated. This is one of the reasons why an action potential cannot be followed too close by another one (refractory period).
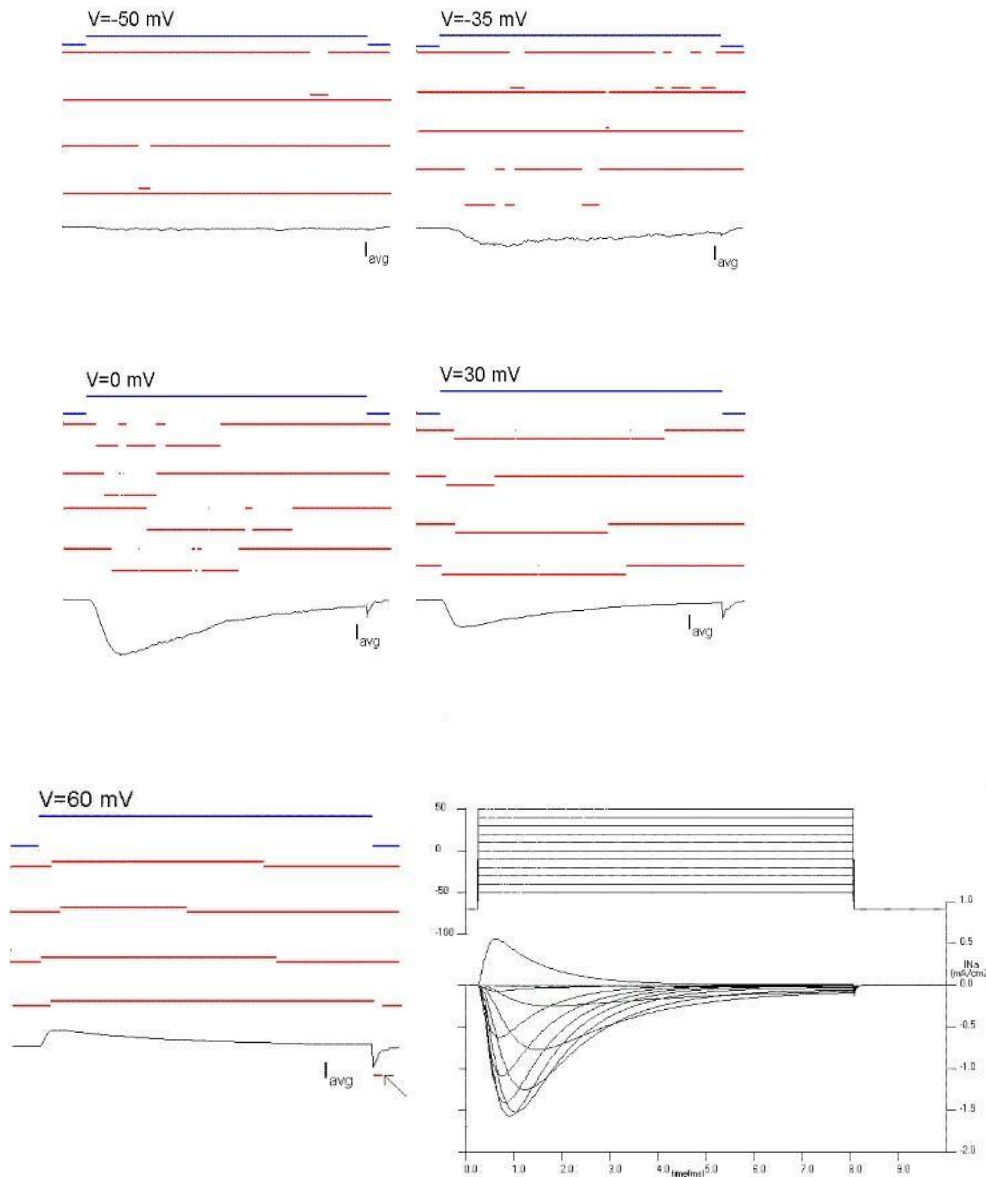

Figure 15. Single sodium channel records and average currents (Iavg) for pulses to -50, -35, 0, 30 and 60 mV starting and returning to -100 mV. Notice that the open probability is very small for pulses to -50 mV although the single channel current is the largest because there is a large driving force. The driving force reverses for a pulse to 60 mV because it is more positive than the Na reversal (equilibrium) potential. Ocasionally, the channel does not inactivate even to the end of the pulse and a single channel event may be observed at the end of the pulse (see arrow for the pulse to 60 mV). The sum of these events produce the ionic "tail" observed at the end of the pulse. BOTTOM (right): family of Na currents for a series of depolarizations ranging from -50 to +60 mV, starting and ending at -70 mV.

NOTES:

4. The Hodgkin and Huxley formulation assumes independence between activation and inactivation giving origin to m^3h. The predictions of the action potential mechanisms are essentially not modified by using independence or coupling between activation and inactivation.
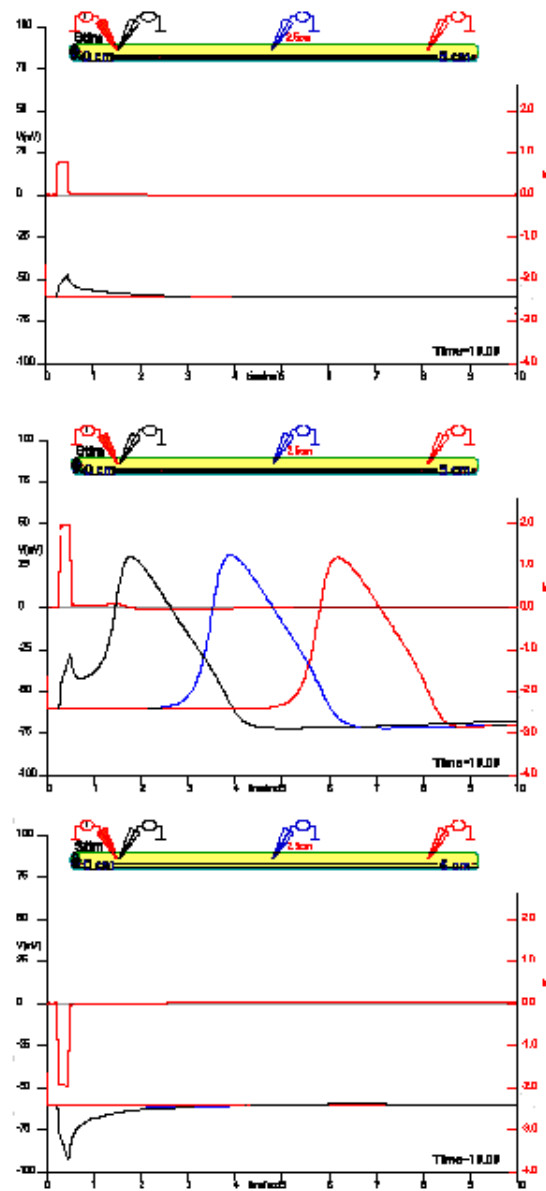
SIMPLIFYING THE AXON.

Figure 16. The response in three different locations of the axon to a variable stimulus at x=0. Top panel: stimulus= 4uA, middle panel: stimulus=10 uA, bottom panel: stimulus=-10 uA.

Figure 16 shows the recording of the membrane potential in three different spots along a squid giant axon in response to several current stimuli at $x=0$. For a current pulse of 4 μA (top panel) the response is local and there is no detectable voltage changes at the second or third electrodes. For a pulse of 10 μA, there is a full action potential response in x=0 and also at x=2.5 and x=5 cm. The responses at 2.5 and 5 cm are similar in time course but delayed with respect to each other: these are recordings of the propagated action potential. A negative pulse of -10 μA produces only a local response in the negative direction and there is no propagation.

From the above, it is clear that the voltage is a complicated function of both time and distance, $V=V(t,x)$**.** But, even worse, it is not a simple function of the stimulus, because there is little response for negative or small positive stimuli and a large voltage response (the action potential) for a larger pulse. This is an explosive process and the study of explosions is difficult unless we can control them.

The first simplification will be to eliminate propagation and this can be achieved in the squid giant axon by inserting a wire along the axis of the axon to make the inside isopotential. This is already an important simplification because with this operation we eliminate the *x* variable and now *V* is only function of time, *V=V(t)*. What does the response look like when there is no propagation? In fact, it looks very much the same. Fig. 16 show the voltage responses ([the Membrane action potential](#)) to four different stimuli, two negative and two positive. The action potential response is only obtained for the large depolarization, as it was the case where propagation was allowed. Our conclusion is that the non-linear voltage response of the membrane to the current stimulus is an intrinsic property of the axon membrane.
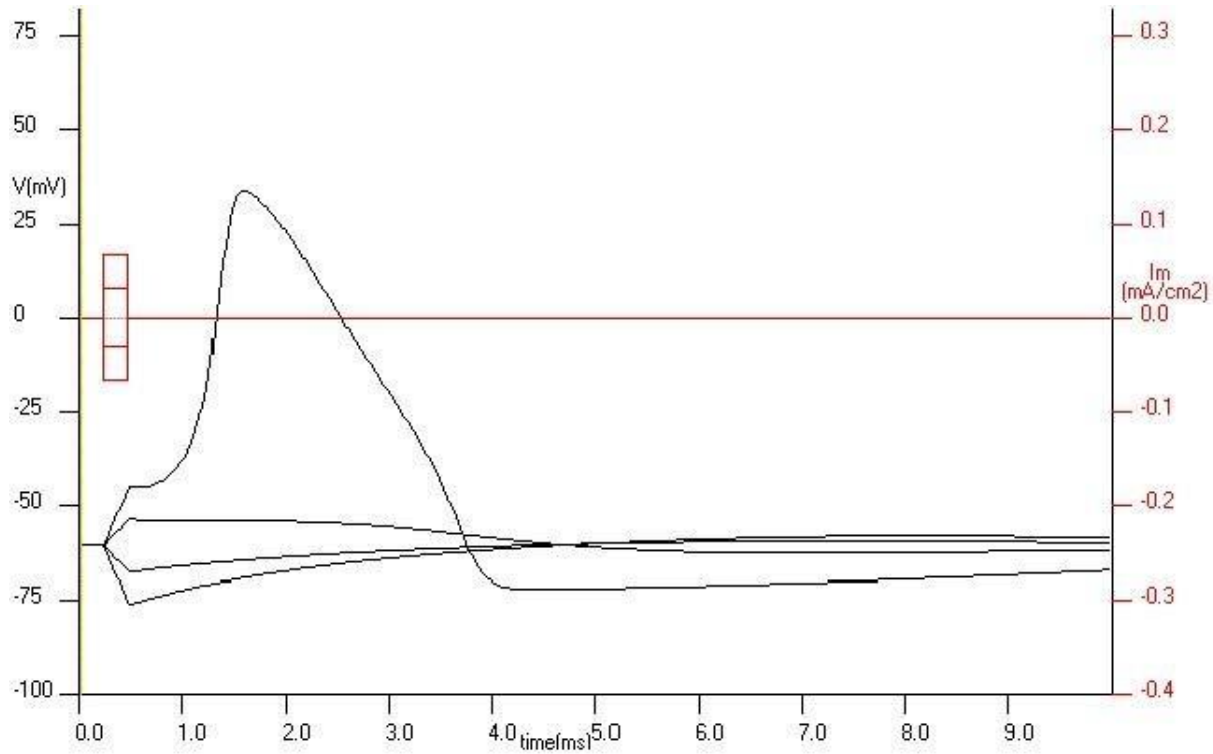

Figure 17. The response of an axon with an axial wire to stimuli of -10, -4.5, 4.5 and 10 uA . Only the stimulus of 10 uA elicited an action potential.

Hodgkin and Huxley decided to study the properties of the axon membrane with *voltage pulses* instead of current pulses to control the explosive mechanism involved in the action potential generation. The technique used to deliver voltage pulses (or any other voltage waveform) to the membrane is called the **voltage clamp** and it was introduced by K. S. Cole.

We have learned that the Na and K channels in the axon change their open probability depending on the voltage across the membrane and in fact the P_o is independent of the current flow. Therefore it is natural to study the properties of the membrane under voltage clamp instead of current stimulation because membrane potential is the variable that controls channel opening.

The second good reason to use voltage control is that the application of a sudden step of voltage will instantly charge the membrane capacitor, eliminating the contribution of the capacitive current in the recording of the membrane current[5].

Summary

Voltage clamp, using voltage steps, is the tool to use because:

-The conductances are voltage dependent and not current dependent

-Constant voltage (immediately after the step change in voltage) will not produce capacitive current

Macroscopic Currents and the voltage clamp

We have studied the properties of single K and Na channels in response to voltage steps and our study was done under voltage clamp because we were imposing voltage steps with the patch pipette across the membrane. This is easily accomplished with the patch clamp technique because, as the pipette diameter is only 1-2 µm, the membrane potential is homogeneous in the patch.
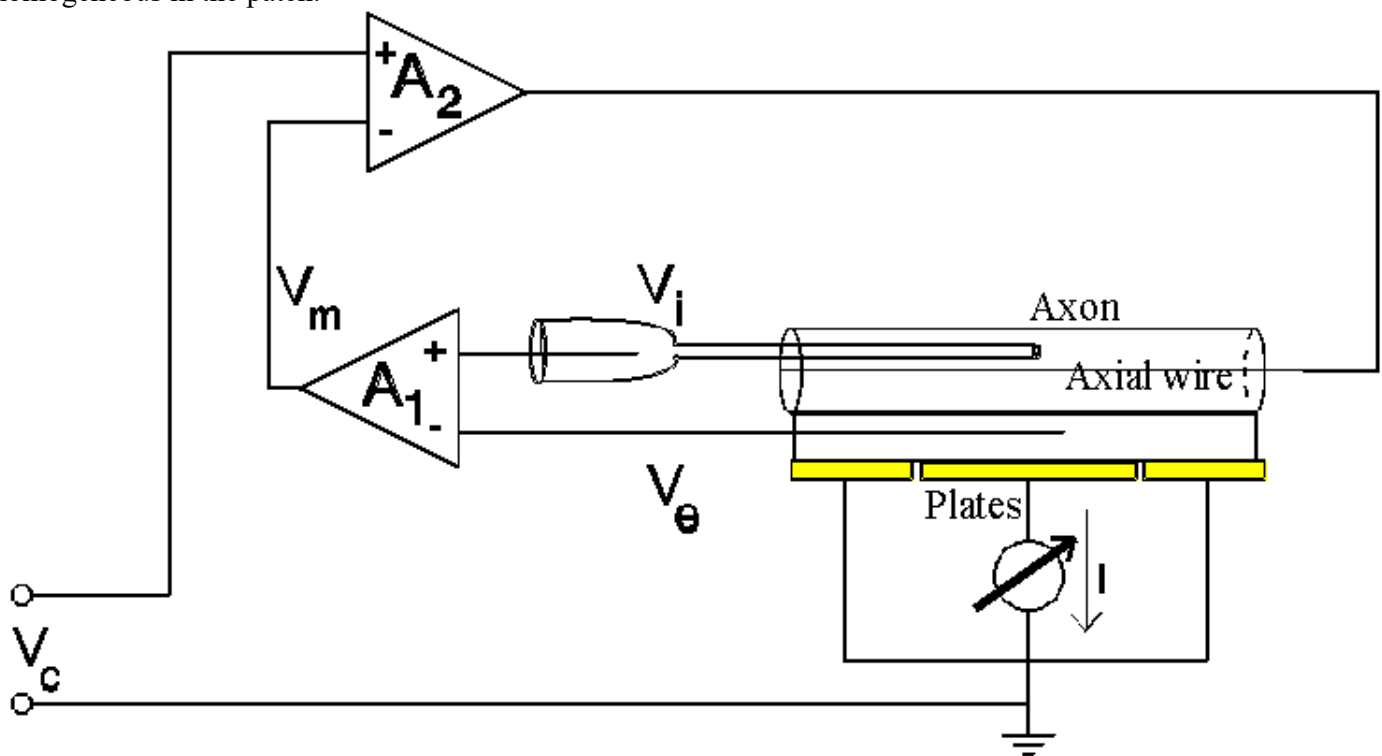
Figure 18. Voltage clamp of the squid giant axon. V_i is the internal potential measured with a pipette inserted in the axon. V_e is the external potential measured by an external electrode. $V_m = V_i - V_e$, as computed by amplifier A_1. A_2 compares V_m with V_c (which is the command voltage) to inject current I, which maintains V_m at V_c. The current is injected by the axial wire and crosses the axon membrane and is drained by the chamber plates and measured by a current measuring device..

A different situation arises if one wishes to study the properties of a population of channels in a large membrane area under voltage clamp, as it is the case of the axon. This is because imposing a voltage in one spot does not guarantee that other regions further away will have the same potential. In fact, as we will see later, the potential in other regions is different.

This problem has been solved in the case of the squid giant axon introducing a wire inside the axon (the black axial wire in Fig. 18) to make the interior of the axon isopotential as we did it before to eliminate propagation. The voltage clamp is achieved using a negative feedback arrangement that compares $V_m=V_i-V_e$ with V_c, the command voltage, to inject the appropriate current I, which will be equal to the membrane current. Therefore, in this technique the experimenter imposes a membrane voltage V_c and measures the membrane current I.

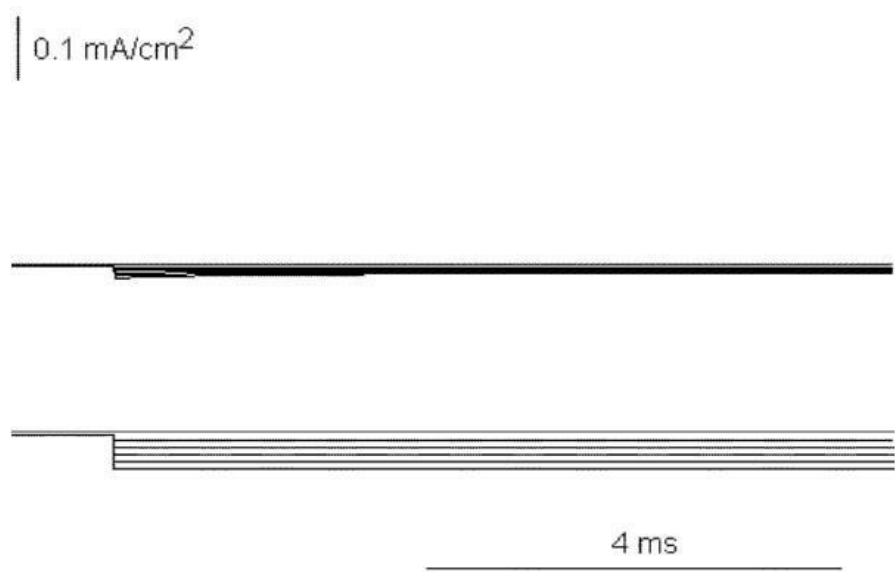

Figure 19. Ionic currents in response to hyperpolarizing pulses from -70 to -120 mV in 10 mV increment.
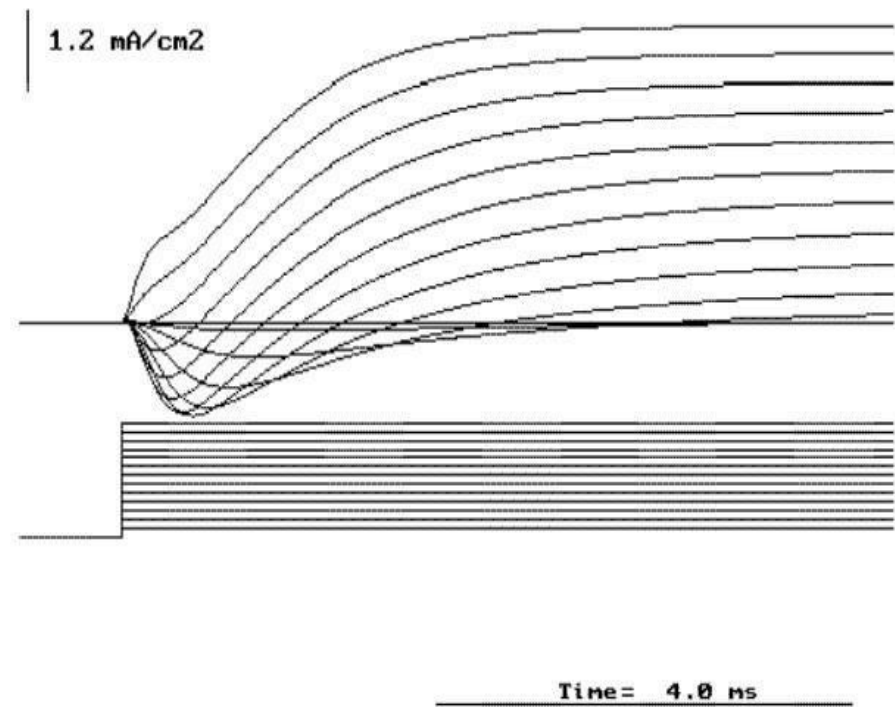

Figure 20. Currents in response to depolarizing pulses from -60 to 60 mV in increment of 10 mV from a holding potential of -70 mV.

The membrane currents during voltage clamp.

If we run a series of voltage steps starting from -70 mV (near the resting potential) we find that for hyperpolarizing pulses (negative going, such as to -90 mV) the membrane current is negative (inward current) as expected, and it grows linearly with the magnitude of the step (see Fig. 19) . In addition, the current through the membrane is practically constant in time, indicating that it behaves close to a passive resistor. For depolarizing (positive going) pulses (see Fig. 20) the same is observed for only very small pulses but for larger depolarizations, the current is much larger (compare the calibration bar in Figs. 19 and 20). The current first is **inwardly** directed and later in the pulse it becomes **outward** (see Fig. 20). The initial inward current is maximum when the potential is about 0 mV and with larger depolarizations becomes smaller. Beyond +40 mV the outward current exhibit a first fast component and then a slower rising and maintained component.

We know from the results of single channel recordings that there are two main types of channels: Na and K. In the case of single channel recordings, the separation of the channels is relatively simple: if there is one channel in the patch, we just observe its characteristics and decide which type it is. When the recording is from a large region of membrane that may contain 10 billion channels, the recorded current will be the average contribution of both type of channels, and the actual current waveform will depend on the proportion of Na and K channels in the membrane. Hodgkin and Huxley separated the two components by changing the ionic composition of the solution bathing the axon (exchanging Na for an impermeant ion).

The giant axon can be internally perfused, allowing the exchange of the internal medium to different solutions of known composition. We will describe now the ionic currents of the axon by controlling the ionic composition on both sides of the axon (Hodgkin and Huxley exchanged the external solution only); this procedure will allow us to separate specific components of the ionic currents. We will identify these currents with the currents produced by K and Na channels whose properties have already been described at the single channel level. The following sections will give an overview of the macroscopic currents, and they will expand on some aspects that were not treated in detail in the sections on single channel properties.
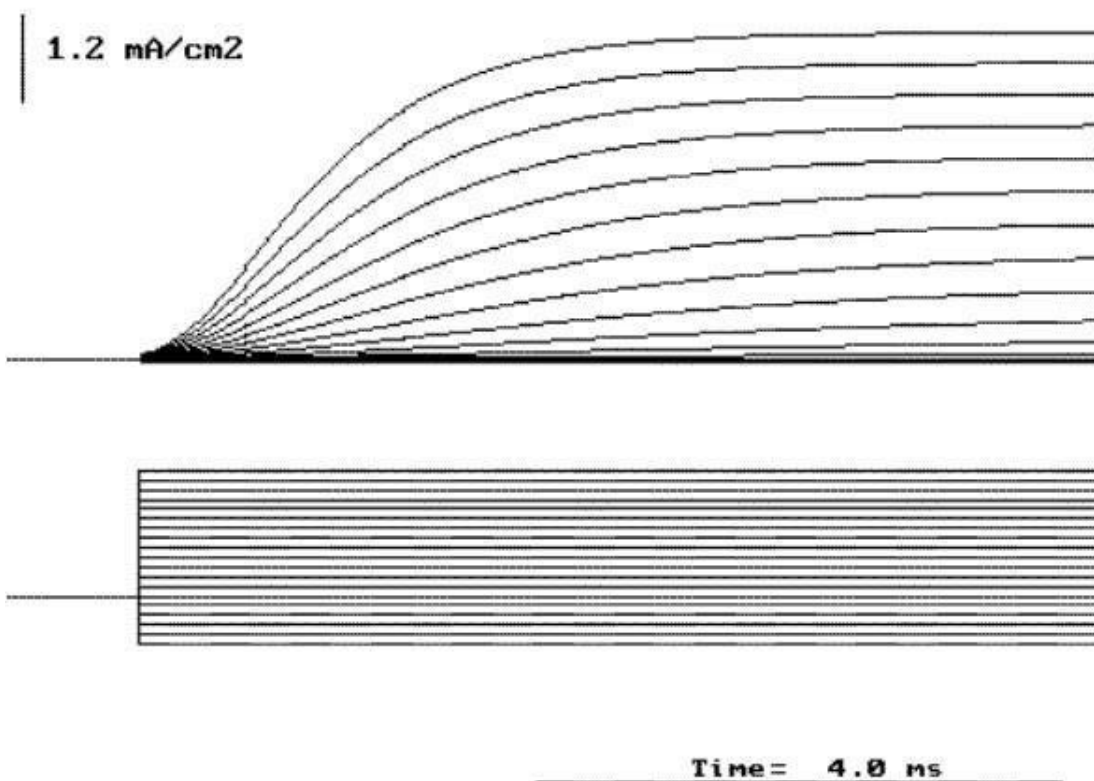

Figure 21. Family of potassium currents elicited by pulses from a holding potential of -70 to +60 mV in increments of 10 mV.

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The Potassium current. We will start by exchanging all sodium ions on both sides of the membrane with the cation choline. This ion was found to be impermeant by Hodgkin and Katz and by Hodgkin and Huxley. In addition we will add a toxin called tetrodotoxin (TTX) which was found to block the sodium conductance. Under these conditions if we perform a voltage clamp experiment as described above, the currents are quite different (see Fig. 21). All the inward components as well as the early outward current have disappeared. The general characteristics of these currents are that they become larger and faster as the depolarization is increased and that they are absent for hyperpolarizing pulses. This is exactly what we found when we averaged many single K channel recordings at several potentials (see Figs. 10, 11 and 12). As expected, the current is outward with depolarization because the internal concentration of potassium is about 40 times the concentration of potassium outside. We can characterize this current by plotting the current at the end of the pulse as a function of the membrane potential during the pulse as shown by the open circles in Fig. 22. where it is clearly seen that the I vs. V relation is very non-linear for depolarizing pulses and practically zero for hyperpolarizing potentials. The open squares in Fig. 22 corresponds to the open channel characteristics scaled by the number of channels, and as expected, it is linear and crosses the axis at the potassium reversal potential (see Fig. 13).

If the potassium inside and outside the axon is replaced by choline, then most of the current disappears, suggesting strongly that it is carried by potassium; the current that remains is very small and is linearly related to voltage, that is, it behaves like a resistor This remaining current was called leakage by Hodgkin and Huxley and plays an important role in the resting potential and in the actual value of threshold for excitation.

The potassium conductance. Given the results of the previous paragraph it is possible to define a quantitative relationship between current and voltage using the concept of conductance. The current I_K is given by the product of this conductance and the driving force: $I_K=g_K(V,t)$ $(V-E_K)$ where $g_K(V,t)$ is a function of the potential V and time t.
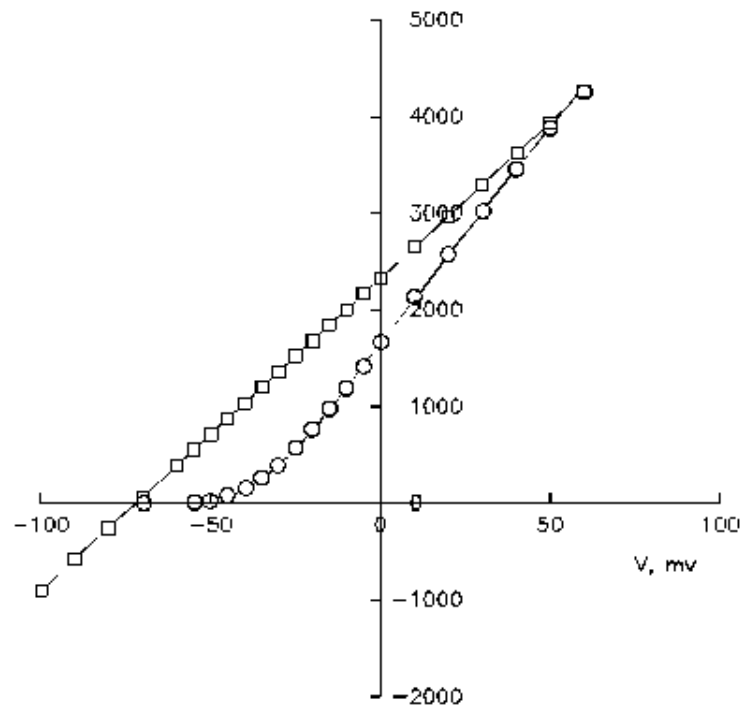
Figure 22. Potassium currents at the end of an 8 ms pulse (circles) and the scaled value of the open channel current (squares).

The detailed analysis of the conductance will require the understanding of its time course and its dependence on voltage. If we take the potassium current traces and divide them by $V-E_K$ both during and after the pulse we have the time course of the conductance for that particular depolarization and its recovery upon repolarization. Examples of the time course of g_K for two different depolarizations are shown in Fig. 23. The important features are:

i) the conductance develops faster at more positive potentials, ii) the conductance

at the end of a long pulse is larger at more positive potentials,

iii) the development of the conductance is sigmoid, that is, it shows a lag before turning on and upon repolarization it turns off without appreciable lag. These features are compatible with a high order process with multiple steps during turning on and a first order process in turning off.

Figure 23. The potassium conductance as a function of time during and after a pulse to -10 and 60 mV.

The correlation of the *n* formulation and single channels. We have seen that the macroscopic ionic currents are the result of a large number of unitary current events through single molecules called ionic channels. In the simplified view of the K channel, the probability of the channel to be open, P_o, is given by n^4 and the maximum conductance corresponds to the conductance of one channel times all the channels available N_K:
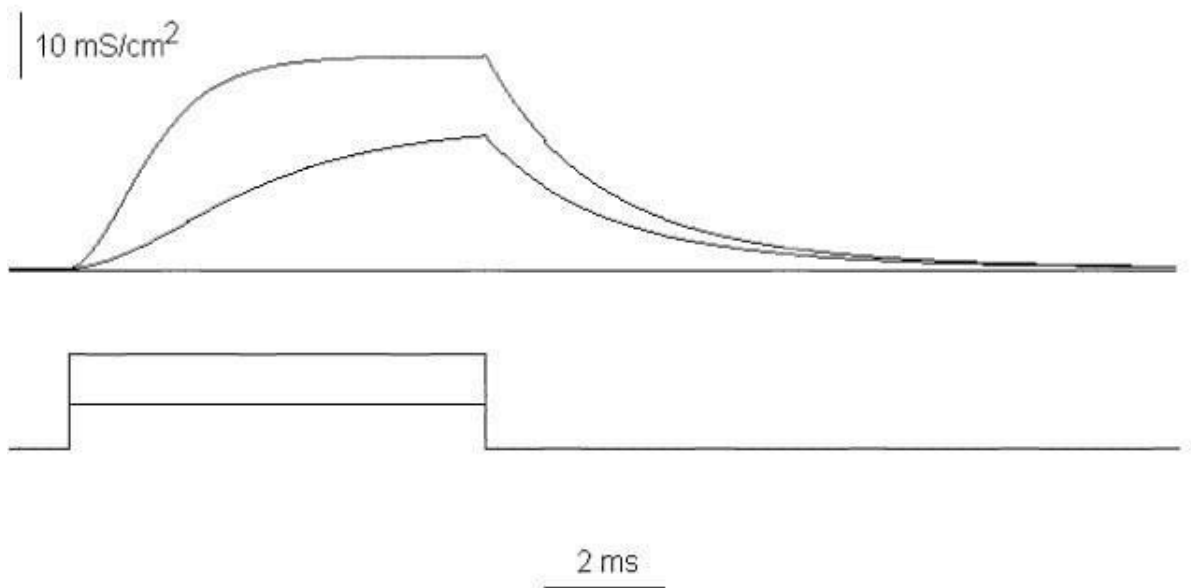
Then $N_K\, g_K\, P_o = g_K(V,t)$ and $N_K\, g_K = g_{Kmax}$. As we saw before, the parameter n is the probability that a subunit is in the active position, therefore n is a function of voltage and time, $n=n(V,t)$ and so will be n^4.

It is important to note that this formulation is able to predict the lag in the conductance turn on (that is, upon depolarization) because all four gating subunits must be in the active position for conduction to occur. As this requires the movement of four subunits it is a fourth order process. On the other hand, the turn off (that is, the repolarization) depends on the return of only one of the subunits which is a first order process.

The sodium conductance. If the axon is perfused and bathed in solutions free of potassium ions, the current recorded for clamp steps are quite different than in the normal solutions as it was shown in Fig. 15. In this series of traces, the holding potential was -70 mV and pulses ranged from -60 to +60 in steps of 10 mV. The currents are inward for small depolarizations and they are not maintained for the duration of the pulse but instead they decrease spontaneously and, as the depolarization is made larger, the turn on and turn off becomes faster. The direction of the current reverses at about 50 mV and this potential coincides with the sodium Nernst sodium potential, as computed with the known concentrations of sodium inside and outside the fiber. If we plot the *maximum (peak) current* as a function of pulse amplitude we obtain the relation plotted as open circles in the top panel of Fig. 24. Notice that the current is inward for all potentials negative of 50 mV and outward when the potential is more positive than 50 mV. In addition, from this plot it is very clear that the slope of the *I-V* relation is *negative* for potentials more negative than about 0 mV. A negative slope in the *I-V* curve means that there is a *negative resistance*, that is, an element that instead of dissipating energy it

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is in fact injecting energy in the system. This energy can be traced to the Na driving force that is forcing sodium ions to flow along their electrochemical gradient. The squares in the top panel of Fig. 24 correspond to the *open channel characteristics* scaled by the number of channels (that is, $N_{Na} i_{Na}$).
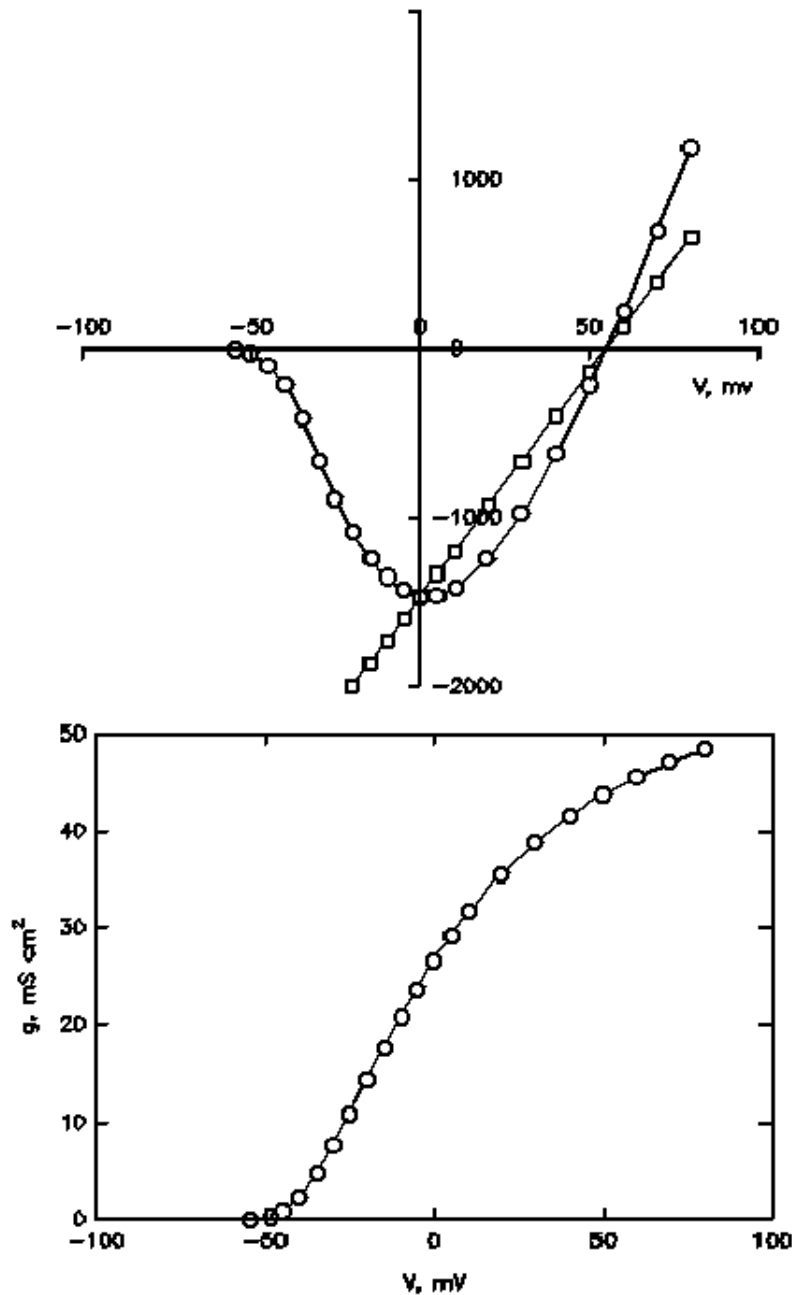
Figure 24. Upper panel: I-V at the peak of the sodium current (circles) and scaled open channel current (squares). Lower panel: peak sodium conductance.

The bottom panel of Fig. 24 is a plot of the peak conductance as a function of membrane potential and it shows the typical sigmoid relation as it was seen for the K channel. This plot was obtained by dividing the peak current by $V-E_{Na}$. (The reader may verify that it can also be obtained by dividing the peak current by the scaled open channel current).

Inactivation of the sodium conductance. When we studied the single Na channel, we remarked that one of the salient features of the sodium current is its decay during the pulse, a process that has been called inactivation. We are going to describe a few experiments that give more insight on the *onset* and *recovery* of inactivation.
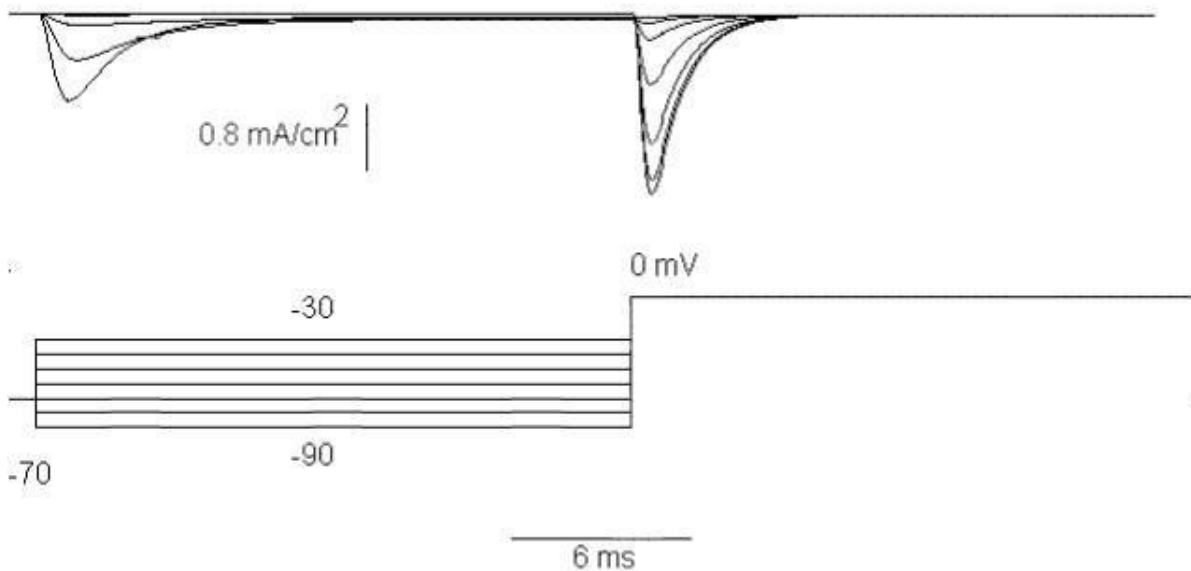
Figure 25. The effect of a long prepulse from -90 to -30 mV on the sodium current elicited by a pulse to 0 mV.

Fig. 25 shows a series of sodium currents elicited by a double pulse experiment. The first pulse is varied between -90 and -30 mV and the second pulse is always to 0 mV. Notice that the first pulse elicits a Na current for depolarizations to -50, -40 and -30 mV and that the amplitude of the current during the second pulse *depends* on the potential during the first pulse. This result is very important because it shows that the current at 0 mV is not always the same but it depends on the previous history: the more depolarization in the previous pulse, the less current elicited by the second pulse. If we plot the normalized current during the second pulse as a function of the value of the first pulse we find a sigmoid curve as shown in Fig. 26.

This curve has been called the h_∞ curve because the inactivation process is controlled by the variable h and the subindex refers to the value of the variable for a very long prepulse. The prepulse effect develops with time; for very short prepulses no channels are inactivated but as the prepulse is made longer the percentage of channels that become inactivated increases. When the prepulse is very long, the effect on the current during the test pulse corresponds to the result obtained in Fig. 25 and shown as h in Fig. 26.

Figure 26. The voltage depndence of the effect of a long prepulse on the sodium current.

```
Total Current [mA/cm2]                              Voltage Stimulus [mV]


 1.0                                                            Time
                                                                40.00
                                                                msec

   0

                                                                50

-1.0                                                            0


                                                                -50



                                    Time [1 msec/division]
```
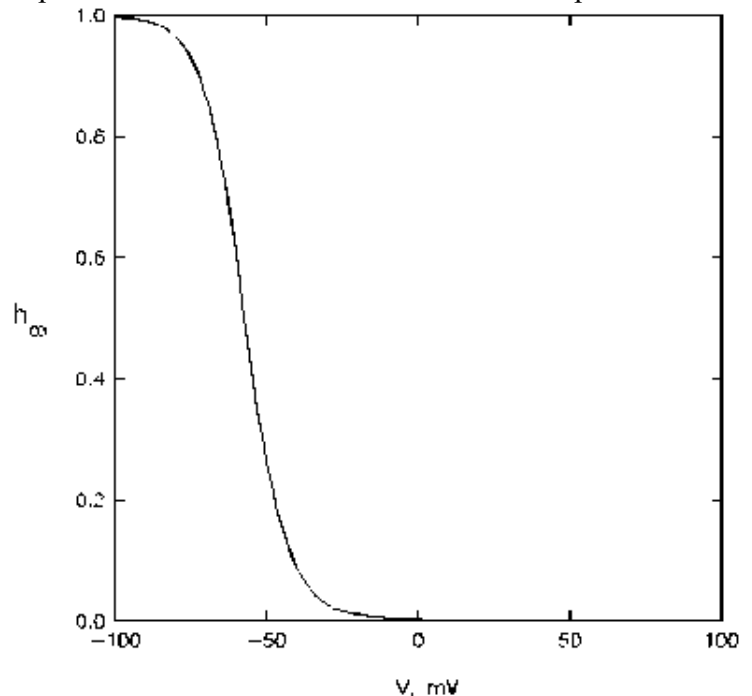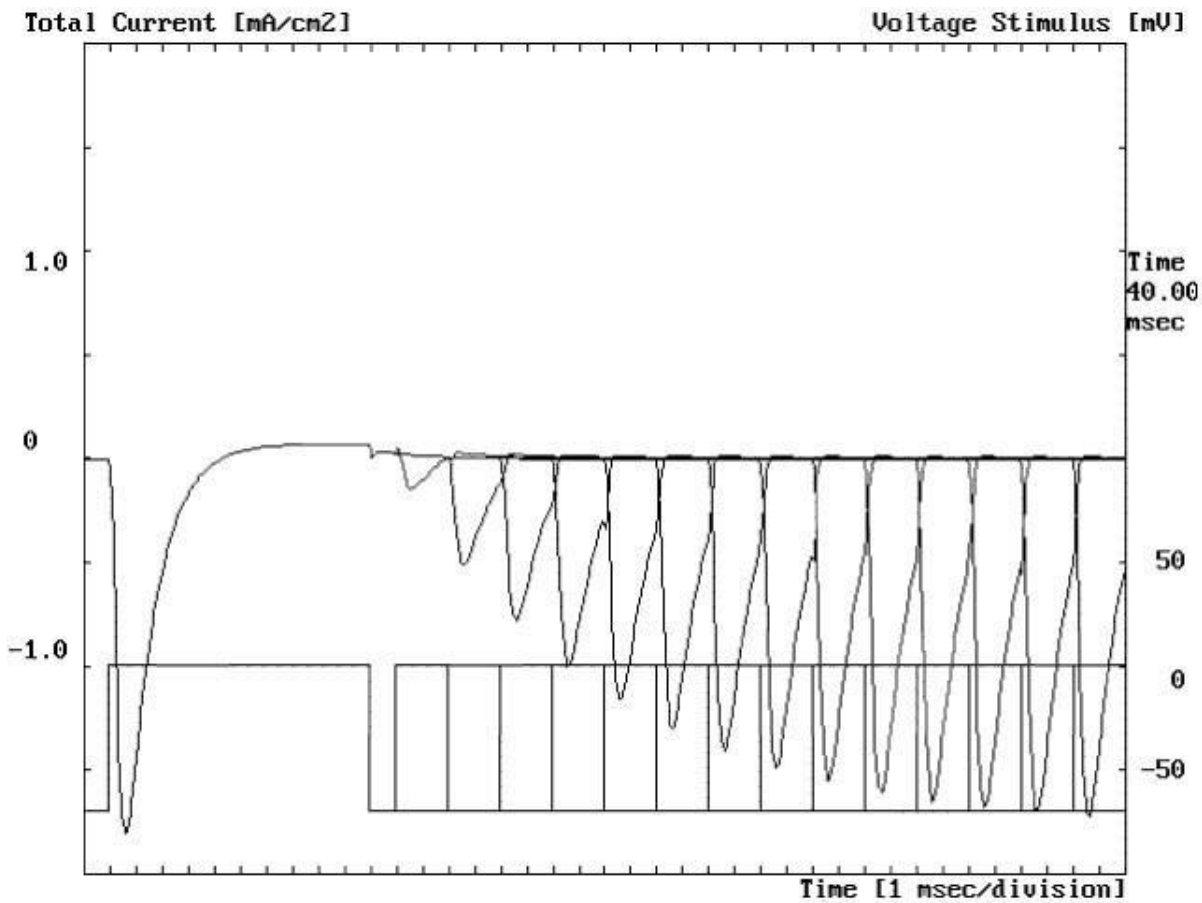
Figure 27. Experiment to determine the time required at -70 mV to recover from inactivation (see text).

Recovery from inactivation. A depolarization opens Na channels and, if maintained, these channels inactivate. A valid question is, how long do we have to wait for the channels to be ready to conduct again? First, it is clear that if the depolarization is maintained, the channel will not conduct because inactivation will persist. We must repolarize the membrane to reset the initial conditions. Fig. 27 shows an experiment to explore the recovery from inactivation. The membrane was pulsed to 0 mV for 10 ms producing an inward Na current. Then the membrane was repolarized back to -70 mV for a variable period of time before pulsing again to 0 mV for a period of 2 ms. The figure shows all the trials superimposed with different recovery intervals at -70 mV. When the recovery interval was 1 ms, the Na current was very small (about a tenth of the current elicited by the first pulse). As the recovery time at -70 mV was prolonged, the peak inward Na current started to increase, reaching half of its original amplitude at about 7 ms and almost fully recovered at about 27 ms (the rightmost current trace shown in the Fig. 27). These results show us that to get all the Na current back we have to return the membrane to its resting value for a period of at least 10 ms. This will have important consequences in the recovery from the refractory period in the action potential.

The leakage current. In addition to the Na and K currents, there is a small current that has essentially a linear dependence on the membrane potential, behaving as a pure resistor with a reversal potential more positive than E_K but more negative than E_{Na}. This current was attributed to non-specific conductances, which grouped together compose the leakage conductance that can be written as

$$I_L = g_L(V - E_L).$$

The capacitative Current.

The [equivalent circuit of the axon membrane](#) includes ionic pathways and a capacitor (see Fig. 2). When the voltage is constant, the current through the capacitative pathway is zero because the capacitor has acquired the charge Q according to the relationship
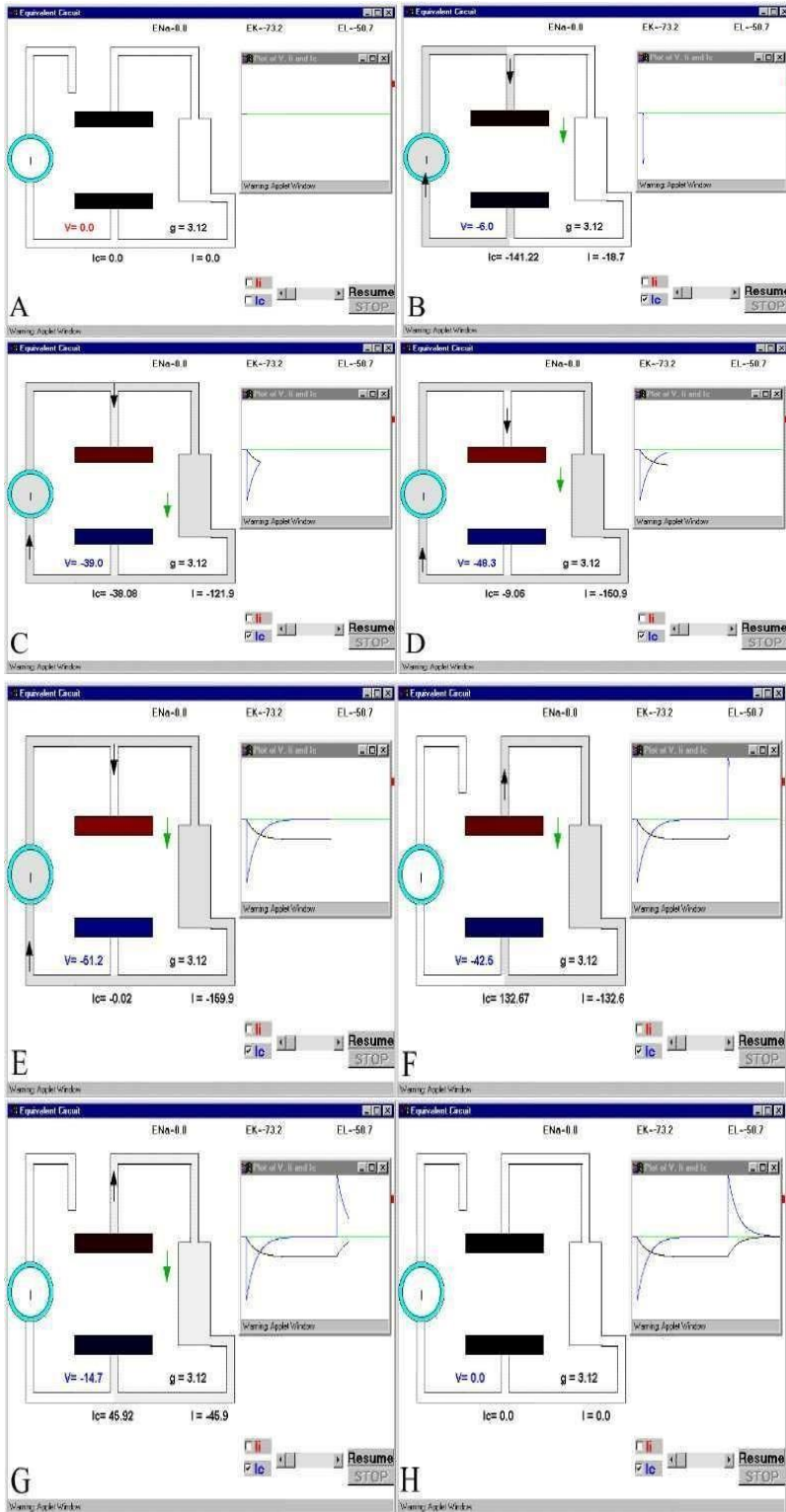
$$Q=CV$$

We have not yet addressed the situation when V is changing because during voltage clamp pulses V is constant (except for the instant when V is stepped up or down). If we are to understand the operation of the axon, we need to consider the situation when V is changing in time because that is exactly what is happening during the action potential.

We must ask first, how does the capacitor acquire the charge Q when the potential is changing from 0 to V? The answer is by allowing a flow of charge (ions) towards the plates (the membrane surfaces) and this flow constitutes an electric current which we will call I_c. We can calculate this current easily if we remember that current is the flow of charge, or the amount charges passing per unit time.

In other words, I_c is the first derivative of Q with respect to time

$$I_c = \frac{dQ}{dt}$$

If we take the first derivative of $Q=CV$, we get

$$I_c = C\frac{dV}{dt}$$

because C has been assumed to be a constant[6]. This equation tells us that as long as V is changing with time, there will be a current flowing towards the capacitor. It also verifies that if V is constant in time, there is no capacitive current.

To get a feeling of the importance of this capacitive current, look at the voltages and currents in the simple membrane circuit of Fig. 2 when we supply current with an external source. In Fig. 28 there is series of pictures that are snapshots of the current circulation in the resistive and capacitive branches after the current generator (the circle with the I inside) is connected and subsequently disconected. The intensity of the current is represented by the darkness in the wires. To the right of each panel, there is a plot of the membrane potential (which is the voltage across the capacitor) and the time course of the current in the capacitive branch.. This is the equivalent of connecting an stimulator and recording the voltage accross the membrane. To simplify the situation even more, let us assume that the membrane starts at 0 mV, that is, there is no internal battery. In Fig. 28A we have our equivalent circuit and a current generator that at present is disconnected from the membrane (the switch is open). The current generator will pass a constant amount of current I (which we will set as inward current) as soon as the switch is closed and the current will be distributed in the capacitative (I_c) and the resistive branch (the ionic channels I_i) as

$$I=I_c+I_i$$

In Fig 28B, we see how initially the current is going mainly into the capacitative branch with almost no current through resistive branch. (The downward spike in the plot corresponds to the capacitive current, which is negative because it is inward). It is clear that at very short times, most of the current supplied by the current generator will go into the capacitative branch because the capacitor was discharged, while the resistive branch will receive much less current.. Parts C and D and E of Fig. 28 show the current and voltage as time progresses. Notice that as the capacitive current subsides, the voltage builds up. At long times (part E), the capacitor has reached its full charge, V has leveled off to its maximum negative value, and consequently I_c becomes zero (Fig. 28 E). If at this point we open the switch, which is equivalent to stopping the current stimulus, then the capacitor will discharge through the resistor and the current in the capacitor branch will flow in the opposite direction. At short times the capacitive current will be very large because V is changing (Fig. 28F) and a large upward current spike reflects the discharging of the capacitor branch. As time goes on (Fig 28G and H) it will subside because all the charge has been redistributed and the energy has been dissipated as heat as the current circulates through the resistor.

The actual time course of the voltage across the membrane is given by solving the above equation of the total I after substituting $I_i=V/R$ and $I_c=CdV/dt$. When the switch is closed, the result is

$$V=IR[1-\exp(-t/\tau_m)], \quad \text{which is the}$$

exponential time course observed in Fig 28.

When we reopen the switch we get

$$V=-IR\exp(-t/\tau_m)$$

In both cases, the speed of charging depends on t_m, which is the product of the membrane capacitance C and the membrane resistance R ($\tau_m=RC$). This product τ_m has been called the membrane time constant. If R or C are small, the charging occurs very quickly, but on the contrary, if they are large, it takes more time to reach the final value.

The generation of the action potential

It is now possible to follow, step by step, the events leading to the potential rise and fall during an action potential. Suppose that we apply a small depolarizing current pulse to our axon with the axial wire (which also prevents propagation). Plate 3 shows a series of snapshots of the electrical equivalent circuit of the membrane including the voltage dependent Na and K channels. The branches are indicated with their reversal potentials and conductances: Na (for sodium), K (for potassium) and L (for leakage). The equilibrium potentials are indicated next to the corresponding batteries. The membrane potential is indicated in the bottom of the capacitor branch, and, as usual, the external side (top, in the figure) is referred as ground or zero potential. To the right bof each panel there is a plot of the membrane
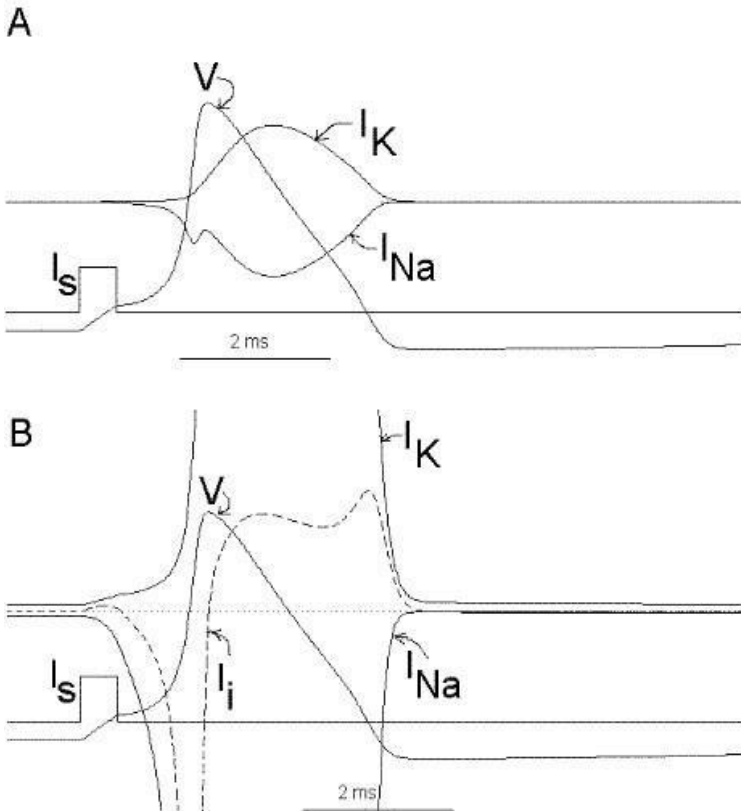

Figure 29. Ionic currents during the impulse. In part Bthe currents are magnified 20 times with respect to part A.

voltage (V) and the total ionic current (Ii). Concomitant with Plate 3 we must look at Fig 29. Fig. 29A shows a plot of the membrane action potential (V) as a result of the stimulating current pulse I_s and the underlying Na and K currents through the membrane. Fig 29B is equivalent to Fig 29A, except that the currents are enlarged 20 times and the total ionic current ($I_i=I_{Na}+I_K+I_L$) is also shown.

In the resting condition, there is no current circulation between inside and outside which means that the sum of currents through all the four branches is equal to zero (Plate 3A and Fig. 29B). We also know that in this resting condition the value of g_{Na} is much smaller than g_K and g_L, therefore most of the current will be across the K branch and the leakage branch. As the resting potential is more positive than E_K, the current in the K branch will be outward and as it is more negative than the value of E_L the current will be inward in the leakage branch. The direction of the currents are indicated by the arrows and their intensity by the darkness in **Plate 3 (Membrane AP in circuit executes by clicking here).** To initiate the action potential, we apply a current pulse directed outward by making the inside more positive. This is shown in Plate 3 as a current generator connected between the outside and inside of the axon when the switch is closed. As we have described above, the first event will be the charging of the membrane capacitance that will occur as most of the current will initially go in the capacitive branch and subsequently through the Na, K and leakage branches in
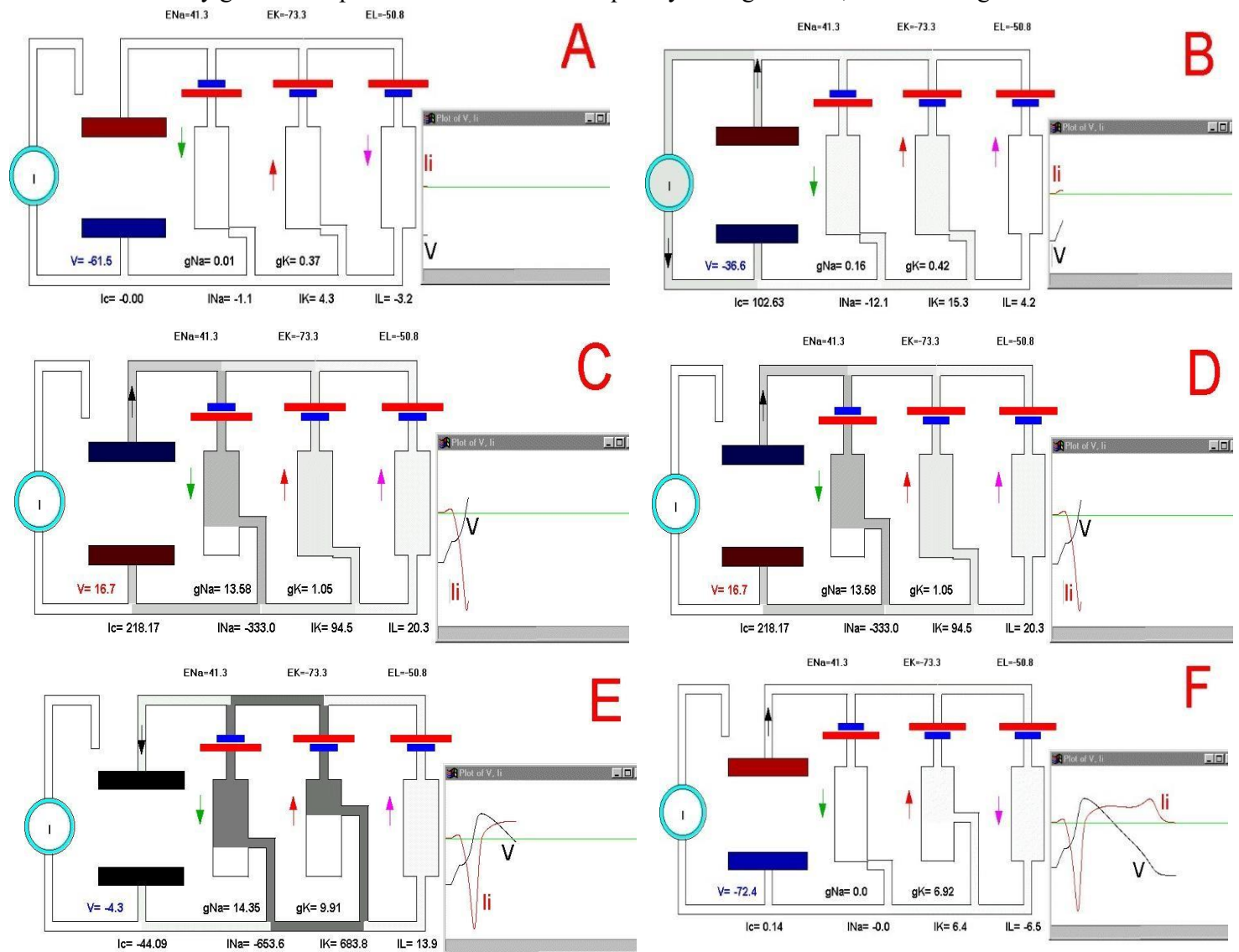

Plate 3. currents and conductances in the equivalent circuit of the membrane during the action potential

proportion to their conductances which will be preferentially through the K and leakage at short times (plate 3B). As the capacitance gets charged, the potential becomes more positive and g_{Na} starts increasing (or R_{Na} decreasing) which produces an inward current through the Na branch. Then the pulse of current is terminated and the total membrane current is again zero. However, there is outward K and leakage current, and inward Na current which keeps increasing until it becomes equal to the sum of I_L and I_K (Fig. 29B). The reason why the inward sodium current increases is because the membrane has been depolarized and the voltagesensitive Na channels have been activated while K channels, which are slower to activate, have not responded yet to the depolarization. This is clearly seen in the trace of I_i shown Fig 29B, which crosses the zero line. At that time, if g_{Na} is still increasing, inward Na current will predominate (see Fig 29B) and as more Na channels open, more inward current will flow which will depolarize the membrane even more, which will open more Na channels, etc (The Hodgkin cycle, Fig. 30). In this fashion, the membrane potential will tend to go to E_{Na}, producing the upstroke of the action potential (Plate 3C). During this time, Na inactivation is increasing due to the depolarization. At the same time the K conductance, slower in turning on, starts increasing to the point that the total ionic current becomes outward through the K branch and the leakage branch, and eventually they cancel the Na current making the total ionic current equal zero (Plate 3D). After this, K outward current predominates and initiates the repolarization phase of the action potential (Plate 3E and Fig 29). Later, g_K predominates pushing the potential towards the value of E_K seen as the underswing of the action potential where g_K is high but the current is low because V is too close to E_K (see Plate 3F and Fig 29).

After some time at this membrane potential, g_K will again become small, producing a return of the membrane potential towards its normal resting value (as in Plate 3A).

There are some important points that must be explained in more detail in this sequence of events. In the resting state, there is no net current through the membrane but there is current circulation through the different branches (see, for example, Plate 3E). The applied pulse of current produces an outward (positive) current through the membrane that results in a depolarization of the membrane. If we neglect the current through g_L and g_{Na}, we find that the voltage across the membrane is the sum of E_K plus the voltage drop across g_K which has the opposite polarity. When the membrane potential has become depolarized enough such that g_{Na} is increased, then the current through the Na branch becomes much larger and inward. As g_{Na} increases, the voltage drop across g_{Na} decreases (V(across g_{Na})=I_{Na}/g_{Na}) and the internal potential tends to be closer to E_{Na}, that is, positive inside. Notice that this is a case where an inward current is producing a depolarization, which is exactly the opposite that occurred during the early phase of depolarization, when the pulse of current was still present. If we look at the *direction of the current in the capacitor branch* of the membrane, however, we see that it is *always an outward current during depolarization and an inward current during repolarization* (Plate 3), as is expected because the voltage across the membrane *is* the voltage across the capacitor, which must be charged positively inside for depolarization, or negatively inside for hyperpolarization.

The Propagation of the Nerve Impulse

Our analysis of the generation of the action potential has been carried out in an axon with an axial wire. This was done to simplify the situation by preventing the propagation of the changes in membrane potential. In fact, with the axial wire, the voltage of the membrane along the whole stretch of axon changes *simultaneously*. Now we have to apply this knowledge to the real axon which does not have an axial wire in it. This means that we have to understand the consequences of the geometry of the axon on the current circulation across the membrane and *along* the axon. To proceed, we will first analyze the properties of a cylindrical axon when we apply very small current pulses. We will first use small pulses to prevent the initiation of the action potential, allowing us to understand the membrane potential changes and current circulation without the complications introduced by the voltage dependent conductances. After we have a clear idea of the current flow and membrane potential changes along the axon will we be in a position to extend the analysis for larger pulses that elicit changes of the Na and K conductances and from there follow the series of events that produce the propagation of the action potential.

Passive properties of a cylindrical axon (Cable properties of the axon).

The simple RC model of the membrane analyzed at the beginning of these notes is only valid provided there are no potential gradients in the inside or outside solutions. Here we are going to extend our analysis to include the geometry of the axon. It is important to realize that this analysis will be first done with a *passive RC* circuit, that is, *with no* voltage dependent conductances. Once we understand this simpler situation, we will incorporate the properties of the channels as we studied them before. Click here to execute an experiment on cable properties of the axon.
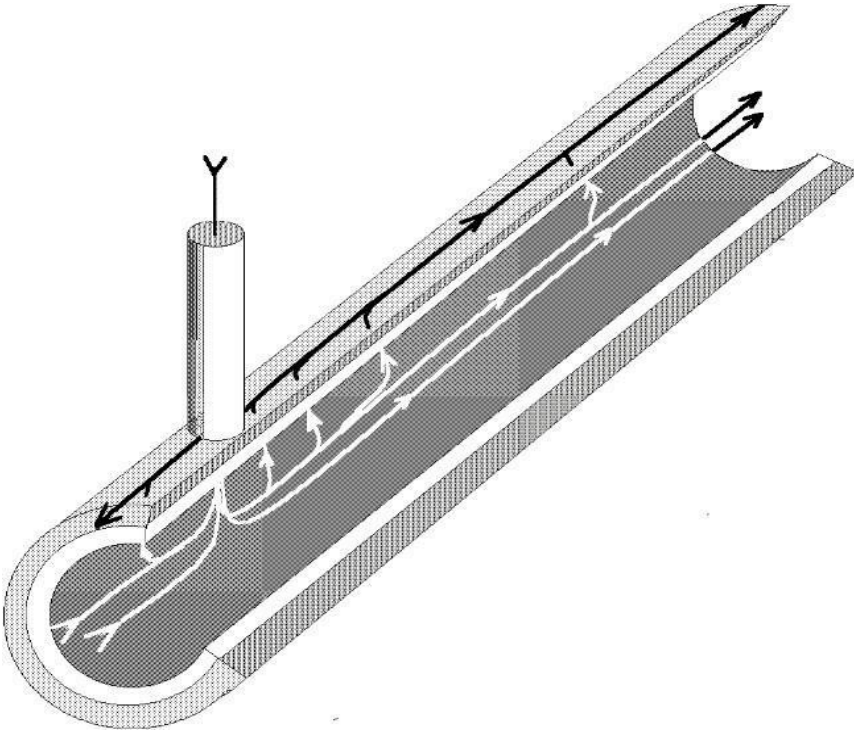
Figure 31. Longitudinal section of an axon showing a few lines of current flow near the electrode impaled at x=0. The other electrode is outside and far away.

Figure 31 shows a portion of an axon immersed in a conductive solution. There is an electrode penetrating the surface which is supplying current with respect to another electrode located outside, and the current circulation is pictured by a few lines of flow. If the membrane were a perfect insulator, no current would flow towards the external electrode. But due to the finite permeability of the axon, some current will circulate longitudinally in the axon interior, and exit through
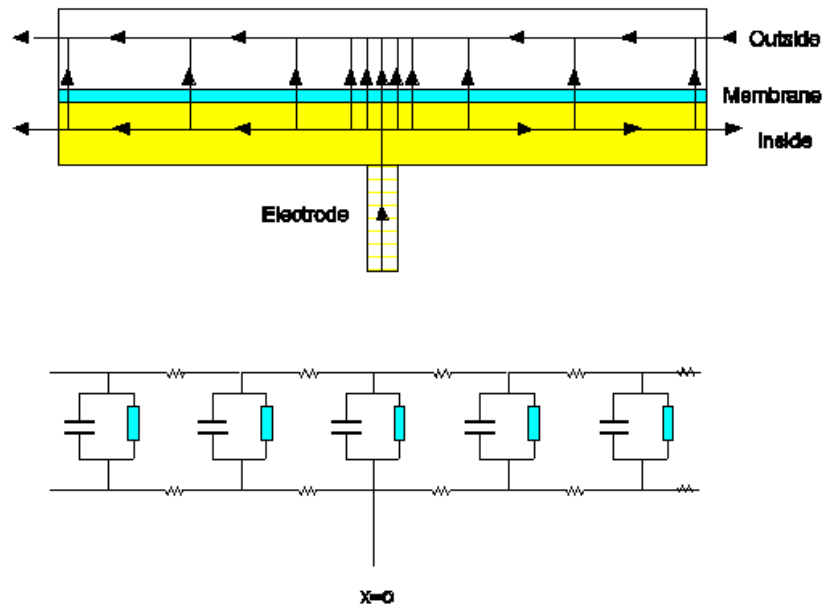
Figure 32. Upper panel: the lines of current flow in the simplified core conductor model. Lower panel: the equivalent circuit of the core conductor model.

35

the surface membrane to be finally collected by the external electrode. It is clear then that due to the current circulation and the resistivity of the axoplasm, there will be **voltage drops** along the axon and none of the two sides of the membrane will be isopotential. Consequently we cannot use our simple membrane model to analyze the membrane potential along the axon.

The detailed analysis of this problem takes into account the geometry in three dimensions plus the time variable; the resulting equation is a partial differential equation in four independent variables. Considerable simplification can be achieved by assuming that all the lines of flow are either parallel or perpendicular to the axon membrane and that there is no potential gradient radially inside or outside the axon (Fig. 32, upper panel). This seems like an arbitrary set of assumptions but it can be demonstrated that it is a good approximation for most of the practical cases. Under these conditions, our model gets reduced to a one-dimensional case with time as the second variable and it can be represented as a series of our membrane elements connected by internal and external resistances as shown in the lower panel of Fig. 32.

To proceed with the analysis, it will be necessary to define several parameters. Let us call r_o the resistance of the outside solution per unit length, given in S/cm. This means that the total resistance on the outside for the segment of length Δx will be $r_o \Delta x$. In the same manner we define r_i as the internal resistance per unit length also in S/cm, giving the total internal resistance of the segment as $r_i \Delta x$. Both the internal and external longitudinal currents will be given in A and the potentials in V. With regard to the currents perpendicular to the membrane we have to consider that the longer the segment the larger is the current, therefore we have to define them per unit length. We will then define the membrane current as i_m in A/cm. In this way the total membrane current will be $i_m \Delta x$. The membrane resistance r_m will be given in Scm, because as we take a longer piece of membrane the total resistance decreases.
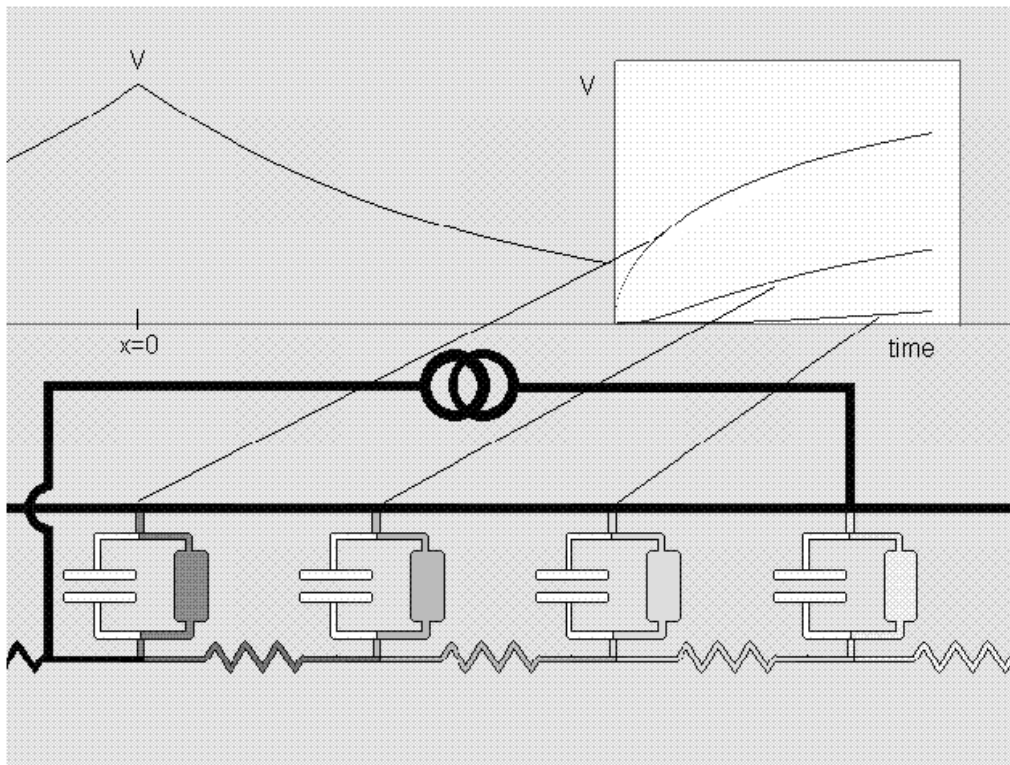
Figure 33. The passive voltage spread in an axon where a current has been injected at x=0. Inset shows V vs time in three spots.

[The analysis of the voltage distribution along the axon as a function of time for a stimulating current step in the center](#) is shown schematically in Fig 33. In this case, the axon is immersed in a large bath of solution, therefore we may consider the external resistance close to zero, which makes the outside essentially isopotential. For this reason, the diagram is showing only the internal resistances connecting the membrane patches. Fig. 33 shows the current intensity as darkness

in the wires, and it also shows the voltage distribution as a function of distance after we have waited a long time and all the capacitors have been charged to their final value (for this reason the currents are only in the resistive branches). When the pulse is suddenly applied, the current will go mainly to charge the membrane capacitance but most of this current will be taken by the capacitance closest to the electrode and much less by the capacitance further away because the internal resistance produces a voltage drop ($V=ir$) and less voltage will be seen by the distant capacitors. This initial capacitive charging may be considered like a short circuit at short times. As the capacitance near the electrode gets charged, the current in that region decreases and more can go to regions farther away and charge the rest of the axon capacitance. This means that regions far away from the current electrode will start increasing their voltage with a time lag. This is shown in the inset of Fig 33 where the time course of V has been plotted at three different points of the axon as indicated by the lines. It is obvious that none of these curves are exponentials as it was the case of the simple membrane model circuit. Even the time course of the voltage in the injection site is non-exponential. The actual function that relates V with x and t is complicated and includes error functions and will not be written here. Considerable simplification is achieved at long times when all the capacitors have been charged. In that case $V(x)$, when t is large, is given by

$$V(x) = \frac{I\lambda r_i}{2}\exp(-x/\lambda)$$

for $x>0$ (replace x by $-x$ for $x<0$). I is the stimulating current and λ is the *space constant* defined as

$$\lambda = \sqrt{\frac{r_m}{r_i}}$$

In the case of high external resistance r_o, the definition of λ includes the sum of $r_i + r_o$ in the denominator. λ represents the distance that one must travel from the injection site to record a voltage that is 0.37 (or $1/e$) the value of the voltage at the injection site. As λ increases, the spread of the voltage change also increases.

The value of λ depends on the membrane resistance and internal resistance. Our resistances have been expressed per unit length. We can compare the value of λ in different cells if we use specific quantities, that is, independent of geometry. Thus $r_m=R_m/2Ba$, where R_m is the specific membrane resistance in Scm^2 and a is the radius of the fiber. Also $r_i=R_i/Ba^2$, where R_i is the specific resistance of the axoplasm in Scm. Replacing these expressions in the equation for 8, we get

$$\lambda = \sqrt{\frac{aR_m}{2R_i}}$$

where
we can see that the space constant is proportional to the square root of the radius.

The ionic events during the propagation of the nerve impulse.
We are finally in a position to explain the propagation of the action potential. We know the details of the relation between membrane potential V and membrane current i at any point along the axon, therefore our task is to put together the relation of i and V at any point with the circulation of currents between different points along the axon.

The events during the action potential propagation (Fig. 34) are similar to the case of the space-clamped (axon with an axial wire) membrane described above, except that the stimulus is not external but is provided by another stretch of axon that has been excited. Suppose that we are considering eight small patches of membrane far from the stimulating electrode which, in turn, is located to the left in Fig. 34. Each panel of Fig. 34 has two parts: the top inset to the right

represents the time course of the membrane potential in the three patches marked blue red and green with traces that are color-coded accordingly. The main region represents the membrane patches with each membrane patch with its capacitance (the long horizontal bars color coded according to the membrane potential), the four membrane current paths: capacitive, Na conductance (in green), the K conductance (in cyan) and the leak conductance (in white). The patches of membrane are interconnected beween them by the resistance of the external medium and the internal resistance of internal medium (axoplasm). The intensity of the currents are represented by the darkness of the conducting path being white when current is zero and black when the current is very high. In the marked patches the currents are indicated numerically.

Now, let us assume that the blue patch (marked blue in Fig. 34) is now becoming excited by current coming from a previous patch from its left hand side (arrows in the axoplasm on the bottom left of Fig 34A). Fig 34A shows the early times when the action potential is far away lo the left of the eight patches shown in the figure: the membrane potential in all patches is the resting potential (-60.8 mV). Sometime later the xcited patches on the left are much closer than to these eight patches (see inset in Fig. 34B). Now the membrane currents of the patches from the left of the figue are making their way via the internal and external resitances into our eigt patches. Notice that the current coming from the left will have an easier path into the blue patch than into the patches to the right because the axoplasm presents resistance to its flow. For this reason the red and green patches are closer to the resting potential and there is no net current in or out of those patches The positive current entering the blue patch will initially flow towards the membrane capacitor (see light gray current path into the capacitor) making the internal plate more positive in the blue patch. In other words, the membrane potential is starting to rise and the membrane is becoming depolarized to -50 mV (Fig 34B). This depolarization (very small at the beginning) becomes more pronounced and starts to turn on Na channels in the blue patch. Once Na channels are turned on, inward current flows through the Na channels but initially most of it goes into charging the internal plate of the capacitor making the potential even more positive in the blue patch (-4.1 mV, Fig 34C). This situation is analogous to the current flow shown in <u>Fig 29B</u> for the case of the space-clamped axon, except that the external current source is replaced here by the Na current from the active (blue) patch. Notice that at this point the increasing current provided by the patch at the left of the blue patch and the sodium currents are both contributing to the depolarization of the red patch (Fig 34B) in to a lesser extent to the green patch. A few tens of microsecond later more of the longitudinal current through the axoplasm reaches now the patches to the right of the blue patch and in fact the red patch becomes much more depolarized. When the Na current of the blue patch increases enough (Fig 34D) the membrane potential has risen to +13.5 mV in the blue patch and is spreading to the other patches. However, the spread is not equal in both directions, because the membrane resistance is different on both sides of the active (blue) patch due to the fact that the patches already traversed by the action potential have been depolarized, with an increase in the K conductance and a decrease of Na conductance due to inactivation. We may consider the most active patch (blue) as a current source with the positive side facing inward.

The Na conductance in the blue patch continues to increase but so does also the K conductance which eventually makes the current through the K channels equal to the current through Na channels corresponding to the peak of the action potential and no current flows from that patch into the the axoplasm (see Fig 34E). At the same time the membrane potential in the red patch is still rising and the potential in the green trace is lagging even more behind as shown in the inset of Fig 34E. Some time later the K current has overcome the Na current and the repolarization phase is initiated. Fig 34F shows when the repolarization is about half way in the blue patch and shows that the red and green patches are lagging behind. Also notice that the current is the axoplasm is flowing in the opposite direction because of the repolarization phase.

In this way, the action potential waveform propagates in one direction and a new action potential will only be possible after the potassium conductance subsides and the inactivation recovers after a few milliseconds of repolarization. In the latest satges, the K conductance predominates in all patches and the membrane keeps repolarizing towards the K equilibrium potential generating the underswing of the action potential. Finally the patches reach the maximum value of the underswing and the leak current starts depolarizing the membrane back to the resting potential while the K conductance shuts off, restoring the initial conditions (not shown).

It is important to realize that the described process of current circulation between patches is continuous and not as discrete as it may appear from the figure. In fact the depolarized region is a large stretch of axon that has no exact

boundaries. Notice that the action potential reaches its peak in the blue patch before it does in the red patch (Fig 34E) and that the repolarization in the blue patch preceeds the red and green patches (Fig. 34F). This means that there is a propagation time between the patches; therefore in a long axon it will take time for the action potential to propagate from the initiating site. The cable properties are important in determining the propagation of the action potential. We have seen that the space constant, λ gives an indication of how far the current passively spreads down along the axon.
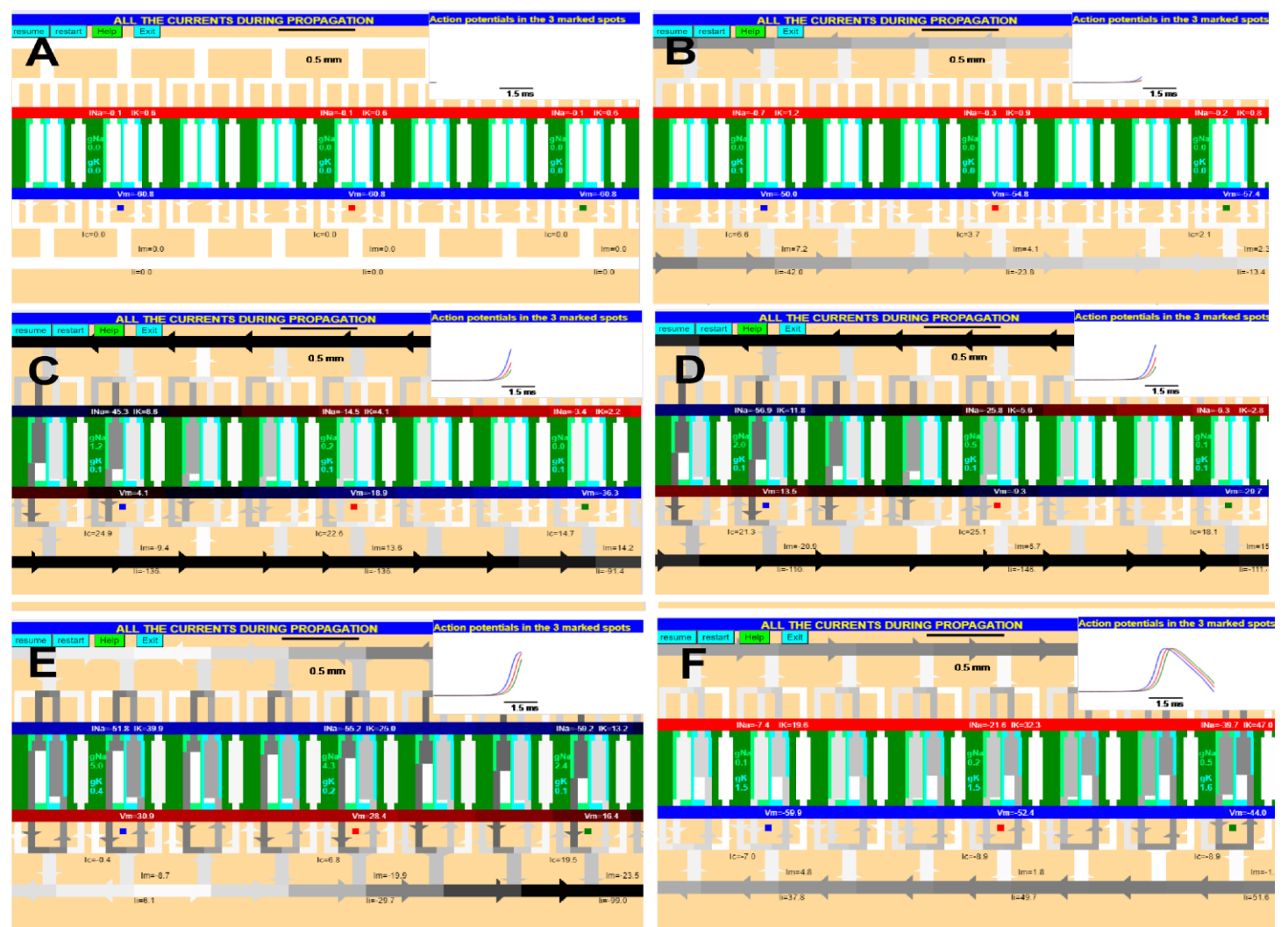

Figure 34. The currents underlying the propagation of the action potential simulated with several consecutive patches. The stimulating electrode is far away to the left of the two pictured patches color-coded red and blue. The time course of the action potential in each patch is shown above each panel and follows the color of the patch. The me Eight membrane patches of a long axon are presented connected between Membrane potential in three of the patches is indicated in internal plate of the capacitor. The currents are shown in each branch and the values of the conductances are shown for the Na conductance (gtreen) and K conductances (cyan) in three of the patches. .

A long space constant will allow the depolarization of the active patch to reach further than a short space constant. Consequently a long space constant will help in a faster propagation of the action potential. Factors that influence λ , such as the internal resistance, membrane resistance or external resistance along the axon will have an influence in the conduction velocity. Remember that the value of l is proportional to the square root of the fiber diameter. If all other factors are maintained constant, such as resistivity, specific membrane conductance and specific capacitance, then we would predict that increasing fiber diameter increases conduction velocity. The giant axon of the squid, which is about 500 μm in diameter, can propagate the action potential at about 18 meter/s, whereas C fibers in vertebrates are around 1 μm in diameter and propagate the action potential at about 1 m/s.

Myelinated Axons

Another strategy for fast propagation is utilized by vertebrates with axons that are covered with and insulating layer (**myelin**) that is interrupted periodically exposing the axon in the **nodes of Ranvier**. In this case, the circulation of current between patches is indeed discrete because there is a very good insulator (the myelin) that covers the axon between nodes of Ranvier. The myelin is formed by a glial cell that envelopes the axon with multiple turns where each turn is just the membrane of the glia. The result is that the internal medium is insulated from the external medium with multiple membranes increasing the membrane resistance (or decreasing membrane conductance) significantly. At the same time, as each membrane is a capacitor, the inside is separated from the outside by many capacitors in series, decreasing the total membrane capacitance significantly. (This is because when n capacitors, each of value C, are connected in series the total capacitance is C/n). The very low membrane conductance and very low membrane capacitance in the internodal region is essentially a passive membrane. The activation of the Na conductance occurs in the nodes where the action potential develops. An active node of Ranvier will excite its neighboring node by the same mechanism outlined above, except that because the nodes are separated by the myelinated region and are so far apart, the actual speed of propagation is increased even when the fiber diameter is not very large. For this reason this type propagation has been called **saltatory conduction**. You can observe the effect of the myelin in an axon by running the Nerve program and selecting the propagated action potential mode. Then, go to AXON PROPERTIES and click on Non-Mielinated (to change to mielinited) and then run the simulation again in either V vs T or V vs X modes. When using V vs X mode you will see why is called saltatory. You can also see how the currents spread between nodes. To start click here.

GLOSSARY

Symbol	Name	Units
γ	single channel conductance	pSiemens (pS)
ϵ	dielectric constant	farad/cm
λ	space constant	cm
τ_m	time constant	ms
A	area	cm^2
a	axon radius	cm
AP	action potential	
c	capacitance	microFarads (μF)
C, C_m	Specific capacitance	(μF/cm^2)
d	distance	cm
E_m	resting potential	mV
E_e	reversal potential (generic)	mV
E_K	K reversal potential	mV
E_L	Leakage reversal potential	mV
E_{Na}	Na reversal potential	mV
e_0	Elementary charge (charge of the electron)	1.6×10^{-19} coul
F	Faraday constant	96500 coul/equiv
G_m	specific membrane conductance	mSiemens/cm^2 (mS/cm^2)
g	specific conductance	mS/cm^2
g_K	Potassium conductance	mS/cm^2
g_L	Leakage conductance	mS/cm^2
g_{Na}	Sodium conductance	mS/cm^2
h	probability of the inactivation gate to be out	-
I	current density	μA/cm^2
i	single channel current	pA
$i_{open\ single}$	current through an open channel	pA
i_m	membrane current per unit length	A/cm
I_c	capacitive current densisty	μA/cm^2
I_i	ionic current densisty	μA/cm^2
I_{Na}	Sodium current density	μA/cm^2
I_K	Potassium current density	μA/cm^2
I_{Cl}	Chloride current density	μA/cm^2
I_L	Leakage current density	μA/cm^2
I_{gate}	Gating current	μA/cm^2
j	Flux	mole cm^{-2} s^{-1}
L	Avogadro's number	6×10^{23} mole^{-1}
m	probability of one Na gate to be active	-
N_K	density of K channels	cm^{-2}
N_{Na}	density of Na channels	cm^{-2}
n	probability of one K gate to be active	-
P_K	Potassium permeability	cm/s
P_{Na}	Sodium permeability	cm/s
P_{Cl}	Chloride permeability	cm/s
P_o	Open probability	-
Q	charge	coulombs
R	Gas constant	8.31 joule $^\circ$K^{-1}
R_i	specific resistance of the axoplasm	ohms cm (Ωcm)
R_m	specific membrane resistance	KΩ cm^2
r_i	internal resistance per unit length	Ω/cm
r_m	membrane resistance per unit length	Ω cm
r_o	external resistance per unit length	Ω/cm
t	time	ms
T	absolute temperature	$^\circ$K
V	membrane potential	mV
x	distance	cm

NOTES:

1. A **potential difference** exists between two points whenever the introduction of a conducting path between the two points would result in spontaneous charge transfer or electric **current**.

2. An electric **capacitor** is a device that allows the separation of electric charge on two conducting plates by a material, or dielectric, that is not able to sustain an electric current.

3. The electromotive force of a battery is the voltage measured from the battery in the absence of current circulation

4. The Hodgkin and Huxley formulation assumes independence between activation and inactivation giving origin to m^3h. The predictions of the action potential mechanisms are not modified by using independence or coupling between activation and inactivation.

5. If the capacitor is ideal and there is no resistance in series, the application of a voltage V will produce an infinite amount of current in an infinitesimally short time transporting a charge q=CV. In practice V is not an step and there is a series resistance, therefore the capacitive current will last a finite time.

6. In the axon, part of the membrane capacitance is a function of voltage due to the gating mechanisms but it is a small fraction that has a negligible effect in the interpretation of the action potential generation.