Foundations I
Fall 2016
J.M. Tepper

IONIC BASES OF THE RESTING MEMBRANE POTENTIAL

I. Physical Bases of The Resting Membrane Potential

A. Structure of the Lipid Membrane

1. Early work suggests that cells possess a surface barrier

a. Microscopic observations
b. Cell loses contents when damaged
c. Intracellular injections of dyes spreads throughout cell but not outside
d. Some types of molecules enter cell from outside but others won't

2. The porous lipid skin model

a. Ernest Overton (~1910)
1. Substances with a high lipid solubility entered cells more easily than substances with a low lipid solubility, and so he suggested that cells were covered with a lipid skin through which the lipophilic molecules could easily diffuse.
2. However, urea and glycerol are not very lipophilic, yet enter cells with ease. Moreover, charged molecules, which are totally insoluble in lipids, manage to enter cells, albeit rather slowly.
3. To account for these observations, Overton proposed that the lipid membrane was full of aqueous holes, and that hydrophilic substances enter cells through these pores.

The Porous Lipid Skin Mode.

a. Experiments with different sized particles suggest that the pores are ~ 6Å diameter
b. The penetration of *uncharged* molecules is governed by their size and *lipid solubility*
c. The penetration of charged substances is governed by their size and *charge*. For weak (organic) acids and bases, the uncharged form usually penetrates cells more readily than the charged form. This has important pharmacological implications, for the fate of drugs that are weak acids or bases often depends on their ionization, which depends on the pH of body fluids.

3. The lipid membrane is two molecules thick.

a. Gorter and Grendel (1925) extracted lipids from red blood cell "ghosts" cells (all lipids in erythrocytes derive from the plasma membrane) and spread the lipid as a monomolecular layer on a trough of water (Langmuir trough). Lipid molecules will be

tightly packed together due to hydrophobicity, each with its hydrophilic end submerged in water, and its hydrophobic end projecting upward. The film covered an area about twice the calculated surface area of the intact erythrocytes, and thus, it was suggested that the membranes consisted of a bimolecular lipid layer, with hydrophobic (nonpolar) ends of molecules from each layer apposed in the center, and hydrophilic (polar) ends directed towards the outside of the membrane.

Additional support for this view has been obtained by measuring the electrical capacitance of cell membranes, that is, the ability of the membrane to hold electric charges separated.

Microscopic observations support the bimolecular model. In the electron microscope, the cell membrane typically appears as a trilaminar structure, about 200-250 Å thick. The outer bands appear dark, and may be the stained protein and polar ends of the lipid. The middle band is light, and may be the unstained nonpolar ends of the lipid molecules.

4. Modern view

The plasma membrane consists of a bilayer of phospholipids whose phosphorylated head groups are oriented towards the aqueous extracellular and intracellular environments. The hydrophobic tails appose each other in the middle. The lipids are free to diffuse laterally - up to 2 µm/sec. (Certain proteins can also move - 50 nm/sec or some 40 times slower)

a. The lipid bilayer is not symmetric

1. The outer lipid head groups contain high proportion of choline glycolipids called *gangliosides* which are glycolipids with *sialic acid* residues. These may play an important role in cell-cell-recognition.
2. The inner head groups contain mostly amino acids. *Phosphotidylinositol* is an important constituent consisting of 2 fatty acids linked through glycerol to inositol. The hydrolysis of phosphotidylinositol constitutes an important intracellular second messenger system that will be described more fully in later lectures.

5. The Majority of the membrane mass is *not* lipid

Chemical analyses of purified membrane fractions have shown that more than 1/2 of the membrane material is protein, and only ~1/3 lipid. Most of the reminder is carbohydrate.

[This is one reason why for visualizing membranes in the EM it is necessary to fix both proteins (with aldehydes) and lipids (with osmium tetroxide).]

The proteins exist in 3 domains, *surface*, *intrinsic* and *cytoplasmic*.

a. Surface Proteins

1. Glycoprotein complex

This consists of shorter sugars projecting into the extracellular matrix from surface proteins. Glycoproteins form the basis for *cell-surface antigens* like the ABO blood group, nerve cell adhesion molecule (NCAM) etc.

2. Proteoglycans

These consist of proteins to which are attached long sugar chains that may be up to 95% of the length of the molecule. The polysaccharide part is referred to as a *glycosaminoglycan*. These are believed to play a role in the cell-cell recognition that takes place during development. As a whole these surface glycoproteins are called the *glycocalyx* and account for the fact that the thickness of the cell membrane is about twice what we calculated on the basis of capacitance.

b. Intrinsic proteins

The intrinsic proteins are buried or interposed within the lipids of the membrane (from which they may extend into intracellular or extracellular space), thus the structure of the membrane is termed a *fluid mosaic*. These proteins fulfill many different functions including serving as receptors or recognition sites for neurotransmitters and hormones and the ion channels themselves. Some also serve as enzymes such as transporters (uptake mechanisms) and ionic pumps. They also serve as anchor points for cytoplasmic proteins.

c. Cytoplasmic proteins

There are a large number of these (most of which are negatively charged at normal body pH). Some, termed structural proteins, interact to form part of a support structure for the cell, termed the *cytoskeleton*.

1. **Actin** - short chain protein

2. **Ankyrin**, bound to membrane proteins

3. **Spectrin -** a **heterodimer** in its minimal form but usually found as tetramer or higher polymer- links actin and ankyrin together underneath the plasma membrane and stabilizes cytoskeleton. Spectrin is found only in cell bodies but another brain-specific *fodrin* is present in axons and dendrites of developing and mature neurons and is believed to play a role in organizing the three dimensional structure of axons and dendrites. It may also play a role in limiting lateral diffusion of receptors and ion channels at subsynaptic sites.

4. **Fodrin** – neuron specific cytoskeletal protein

Thus, the membrane is essentially a thin lipid sheath shot through with proteinaceaous aqueous channels.

B. Resting Membrane Potential

Most of you probably already know that the resting membrane potential arises from an unequal distribution of ions across the semi-permeable membrane of the cell. To dig a bit more deeply, we would like to know how the unequal distribution of ions comes about in the first place and how it is maintained.

1. Principle of Electrical Neutrality

The principle of electrical neutrality (PEN) simply states that the total anion concentration in a solution must equal the total cation concentration. This is simply a quantitative statement

of something that may be intuitively obvious: namely, that solutions cannot be charged. Thus, both the intracellular and extracellular environments must be electrically neutral. Clearly, this contradicts something that we all take for granted, namely, that the resting membrane potential arises from an unequal distribution of certain ions across the cell membrane and causes the inside of the cell to have a negative charge relative to the extracellular fluid. As it turns out, the PEN is only approximately true, but in the real world its violation is very minor, because *electrical forces are very much (>10 ^{18} times) stronger than diffusional forces*. Thus, only a tiny charge disequilibrium accounts for large membrane potentials, as discussed below.

2. Gibbs-Donnan Equilibrium (Donnan ,1924)

a. Consider a situation in which a membrane permeable only to H_20, K^+ and Cl^- (a *semipermeable membrane*) is placed into a beaker of H_20, dividing it into two regions.

b. Case 1: Dump some KCl into one side of the beaker. The two ionic species (K^+ and Cl^-), both *permeant*, will distribute themselves equally across the semipermeable membrane. This will preserve the PEN. Since both ions can move, there is no change in the osmotic pressure, and no *net* movement of H_20 occurs.
[Some terminology: membranes and barriers that allow diffusion of a substance are said to be *permeable* to that substance; the substance itself is said to be *permeant*.]

c. Case 2: What if we add a non-permeant anion on one side of the membrane? The non-permeant anion will be associated with an equal concentration of cations (K+) to preserve the PEN. Now the concentration gradient is upset, and K+ will diffuse out to the other side, accompanied by Cl- to preserve PEN, and water will flow into the side with the non-permeant anion to balance osmotic pressure. Donnan showed that this diffusion will continue until:

$$[K^+]_{out}/[K^+]_{in} = [Cl^-]_{in}/[Cl^-]_{out}$$

or

$$[K^+]_{out}*[Cl^-]_{out} = [K^+]_{in} *[Cl^-]_{in}$$

This is referred to as the *Gibbs-Donnan equilibrium*.

d. However, at this point although the system is in electrical and chemical equilibrium, it is not in osmotic equilibrium. If nothing is done, in a closed structure (like a cell), the cell will swell and expand.

3. Control of Cellular Volume

There are (at least) three possible ways in which a cell, faced with osmotic imbalance, could control its volume, and examples of each kind can be found in nature.

a. Make the cell membrane impermeable to water, as well as to the internal solute molecules. - However, this would create many new problems for the cell; growing cells, for instance, must have a way of accumulating water as they grow, and therefore must

have surface membranes permeable to water. There are some special kinds of cell membranes that do exhibit extremely low water permeability (distal tubules and collecting ducts in mammalian kidneys have low water permeability in the absence of ADH). In general, plasma membranes are quite permeable to water (though diffusion of all substances through the most permeable of biological membranes is still many thousands of times slower than diffusion in a free solution.)

b. Applying a countering hydrostatic pressure. - The cell membrane could be composed of, or surrounded by, a rigid nondistensible material in order to prevent swelling. Plant cells and bacteria are protected in this way with a rigid cell wall composed of cellulose. How strong must a cell wall be to balance the osmotic pressure? To illustrate the kinds of forces involved, a container of pure H_20 will support a column of 1 M glucose (from which it is separated by a glucose impermeable membrane) approximately 900 feet high! To construct walls capable of withstanding such forces around every cell would greatly limit cellular motility and shape, and so it is not surprising that this has not proven to be a very workable solution in highly developed organisms to the problem of volume control.

c. Balance the osmotic pressure - The solution in animal cells has been to balance osmotic pressure osmotically, by adding a non-permeant ion (Na^+) to the extracellular environment in order to balance the increased pressure that arises from the excess electrolytes in the cell.

II. Ionic Basis of the Resting Membrane Potentials

A. Resting Membrane Potential - We can define resting the membrane potential (V_m) as the difference in potential between the inside and the outside of the cell:

$$V_m = V_{in} - V_{out}$$

V_m is generated by a differential distribution of ions across the cell membrane due to its selective permeability.

B. Ionic Concentrations Across the Squid Giant Axon

	Squid Giant Axon	
	in	**out**
K+	**400 mM**	**20 mM**
Na+	**50 mM**	**440 mM**
Cl-	**52 mM**	**560 mM**
proteins-	**385 mM**	**0**

1 Permeant ions will diffuse through the plasma membrane down their concentration gradient through special pores or channels creating a charge imbalance that results in a

potential difference across the membrane. How can we quantify this diffusion, and what are the limiting conditions?

C. Equilibrium Potentials

1. At equilibrium, diffusion in = diffusion out

Flux is proportional to concentration, so for movement of K^+:

$$\textbf{flux}_{\textbf{in}} = r_{in}[K]_o$$

where r_{in} is the rate constant for inward movement
similarly:

$$\textbf{flux}_{\textbf{out}} = r_{out}[K]_i$$

Thus

$$\textbf{net flux} = r_{in}[K]_o - r_{out}[K]_i = pK$$

If the membrane were not charged, r_{in} would equal r_{out} which would equal the permeability, ***p*K**,
where *p*= rate of net movement of ion

$$p = \text{net flux} = r_{out} = r_{in}$$

Since $[K]_i$ is 20 x $[K]_o$, the net outward flux of K would be 20 times the inward flux. However, since the membrane is negatively charged, potassium moves out more slowly than it moves in. Because of this, the two rate constants differ, depending on valence (charge), membrane potential as well as on concentration.
It can be shown that:

$$r_{in} = p\, zV'/(e^{zV'}-1)$$

where z= valence (1), V' = membrane potential divided by the thermodynamic potential (RT/F) or $\mathbf{V'=V_m/(RT/F)}$, T is the absolute temperature and F is the faraday constant (96,501 coulombs/mole)

(RT/F is the potential needed to balance an e-fold concentration ratio across the membrane) = 25 at room temperature.)
thus, **V'=Vm/25**

For example, this means that for a cell at -75.46 mV:

$$V' = -75.46/25 = -3.02$$

and

$$e^{-3.02} = 0.0488$$

so

$$r_{in} = -3.02p/(0.0488-1)$$

$$= 3.175p,$$

i.e., the inward flow of a positively charged ion is more than 3 times greater than if there was zero membrane potential

similarly for an outward movement:

$$r_{out} = p_k zV'/(1-e^{-zV'})$$

and

so

$$r_{out} = -3.02p/(1-20.49)$$

= 0.1546 or 1/0.1546 = 6.45 times smaller than if the membrane were not charged

[note that the product of these two correction factors (6.45*3.175)=20.5, or the ratio of $[K]_{in}/[K]_{out}$ from the values for squid axon]

What conditions obtain at equilibrium, i.e., when there is no net flux? This is the same as saying that the net $flux_{in}$ = net $flux_{out}$.

Rewrite net flux using solutions for r_{in} and r_{out} derived above (K stands for the concentration of a permeant ion):

$$\text{net flux} = \frac{zV'}{e^{zV'}-1} p_k K_o - \frac{zV'}{1-e^{-zV'}} p_k K_i$$

multiply numerator and denominator of second term by e^{zV} to get:

$$\text{net flux} = \frac{zV'}{e^{zV'}-1} p_k K_o - \frac{zV' e^{zV'}}{e^{zV'}-1} p_k K_i$$

and rearrange

$$\text{net flux} = \frac{zV'}{e^{zV'}-1} p_k K_o - \frac{zV' e^{zV'}}{e^{zV'}-1} p_k K_i$$

at equilibrium, net flux is zero which means that

$$K_o = K_i e^{zV'}$$

thus at equilibrium,

$$\frac{K_o}{K_i} = e^{zV'}$$

and taking the natural log

$$zV' = \ln\frac{K_o}{K_i}$$

substituting **$V'=V_m/(RT/F)$** back into the equation gives

$$\frac{zV_mF}{RT} = \ln\frac{K_o}{K_i}$$

rearranging to solve for V_m to give

$$V_m = \frac{RT}{zF}\ln\frac{K_o}{K_i}$$

Note that we could also get there from the Boltzman equation which describes the probability of finding a particle in state 1 or state 2 in terms of the energy difference of the two states:

$$\frac{P_2}{P_1} = \exp\text{-}\left(\frac{u_2\text{-}u_1}{kT}\right)$$

where P is the probability and u is the energy of state 1 or state 2

Recasting this relation by changing the probability of finding a particle in one state or the other to its concentration and the energy to molar energy (U) gives:

$$\frac{c_2}{c_1} = \exp\text{-}\left(\frac{U_2\text{-}U_1}{kT}\right)$$

Taking the natural log gives:

$$\ln\frac{c_2}{c_1} = \frac{U_2\text{-}U_1}{kT}$$

For an ion with valence z, $U_2\text{-}U_1=zF(E_2\text{-}E_1)$, thus

$$E_1 - E_2 = \frac{RT}{zF}\ln\frac{c_2}{c_1}$$

since $V_m=E_m=E_1\text{-}E_2$,

$$V_m = \frac{RT}{zF} \ln \frac{c_2}{c_1}$$

This equation is called the *Nernst Equation*, and gives the potential at which the diffusional force acting on a *single ionic species* is exactly balanced by the electrostatic force of the membrane potential that such diffusion creates. This potential is referred to as the *equilibrium potential*. At T=20° C(room temperature), the Nernst Equation simplified to:

$$E_m = \frac{58}{z} \log \frac{[x]_o}{[x]_i}$$

At normal body temperature for mammals, (37° C), this becomes :

$$E_m = \frac{62}{z} \log \frac{[x]_o}{[x]_i}$$

Thus, for K+, using the data from the squid axon above, the equilibrium potential would be

58 log(20/400)=58 log(0.05)=58*(-1.301)= -75.46 mV

We can calculate similar equilibrium potentials for the other permeant ions:

	Squid Giant Axon		Equilibrium potential
	in	**out**	
K^+	**400 mM**	**20 mM**	**-75mV**
Na^+	**50 mM**	**440 mM**	**+55 mV**
Cl^-	**52 mM**	**560 mM**	**-60 mV**
Ca^{2+}	**10^{-4} mM**	**10 mM**	**+125 mV**

	Typical Mammalian Cell		
K^+	**140 mM**	**5 mM**	**-89.7 mV**
Na^+	**15 mM**	**145 mM**	**+61.1 mV**
Cl-	**4 mM**	**110 mM**	**-89 mV**
Ca^{2+}	**10^{-4} mM**	**~2.5 mM**	**+136 mV**

There are a few points worth reviewing here: Although the PEN is violated (how and why?), the violation is not very great since the electrostatic force is so much stronger (>10

times) than the diffusional force. For a cell with a resting membrane potential of -80 mV, for every 100,000 intracellular cations, there are ~100,001 anions. [A net difference of 600 charges/μm^2 yields 10 mV change in potential]

[JUST HOW LARGE IS THE VIOLATION OF THE PRINCIPLE OF ELECTRICAL NEUTRALITY AT REST ANYWAY?

Recall that **C=Q/V** or Q=CV and that C= 1 $\mu F/cm^2$ for neuronal membrane. Consider a section of squid giant axon 1 cm in length with a diameter of 1 mm. It has surface area of 0.3 cm^2 and thus the total capacitance is 0.3 μF. At a resting membrane potential of -70 mV, the total charge separation is:

$(3 \times 10^{-7}\ F)(70 \times 10^{-3}\ V) = 2.10 \times 10^{-8}$ coulombs

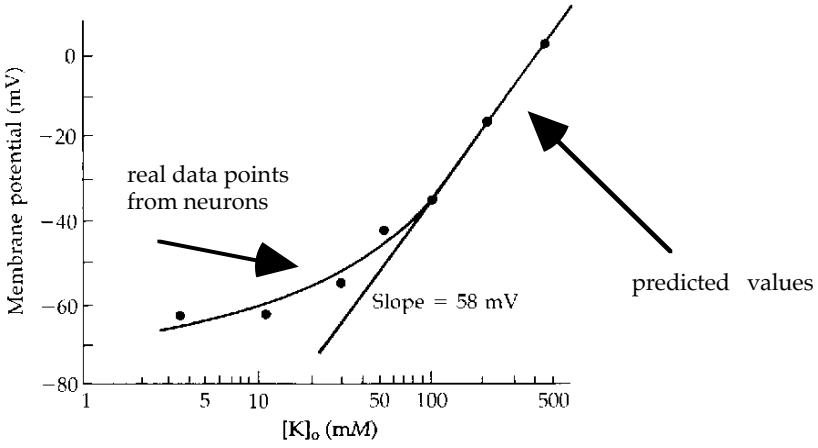
Divide by the faraday constant (96,501 coulombs/mole) to get
2.18×10^{-13} moles of charge.

Dividing this by the volume of the 1 cm piece of axon (7.5×10^{-6} l) gives
2.18×10^{-13} moles/7.5×10^{-6} liters = 2.91×10^{-8} M

Since the internal potassium concentration is approximately 400 mM, or 4x10 -1M, this represents $2.91 \times 10^{-8}/4 \times 10^{-1} = 7.3 \times 10\text{-}8$ of the internal potassium concentration

But (you might say) this is a very large squid axon. However, even in a fiber only 1 μm in diameter, a typical size for CNS axons, the fraction is only about 1000 times larger, still less than 1 part in 100,000. The fraction is intermediate for neuronal cell bodies.]

Thus, the net charge separation is only a tiny fraction of the total charges inside and outside the cell. And here is a really critical point to remember: the charge disequilibirium only occurs **within ±1 μm** of the membrane.



Under conditions like this, the membrane potential is an equilibrium state. It requires no energy input and will stay like this forever. However, as we shall see, this **does not** accurately describe the conditions underlying the resting membrane potential in a real cell.

2. J. Bernstein (1902) On the basis of the Nernst equation, Bernstein proposed that the resting membrane potential was due to selective K^+ permeability. One test of this would be to alter the external $[K^+]$. [Internal $[K^+]$ was first altered in squid axon by Baker, Hodgkin & Shaw, 1962.]

When $[K]_o$ is altered, we see that the membrane potential follows the Nernst prediction for higher extracellular concentration of K^+, but at lower, yet still physiological concentrations, there is a significant deviation from the Nernst potential in the positive direction. It turns out that cells are not completely impermeable to sodium, even at rest [Bernstein was wrong, but he was close]. *Most* of the resting conductance is due to K^+ ions, but some other ionic species are also permeant, e.g., Cl^-, and to a lesser degree, Na^+ and Ca^{++}.

Chloride has very little effect on membrane potential, even though its resting permeability (that doesn't change much during an action potential) is about 10x higher than that of sodium. This is because it is usually passively distributed according to membrane potential. That is, its equilibrium potential is at or close to the resting membrane potential. [see below]

Altering $[Na]_o$ has virtually no effect on membrane potential except at relatively low but physiological $[K]_o$, then observed E_m is more positive than predicted value, i.e., resting membrane potential is more positive than E_K. This is due to very low but still significant g_{Na}. Thus, there is a steady efflux of K^+ from inside of cell and an equivalent influx of Na^+, just large enough to balance the K+ efflux *even at rest. This means that when the cell is at its resting membrane potential, it is in a steady state, not in equilibrium. That is, the cell leaks.* If not compensated for in some way, this steady leak will cause the membrane potential to run down as the intracellular concentration of K+ decreases and that of Na+ increases.

3. Na^+ - K^+ ATPase - Hodgkin and Keynes (1955) filled a squid axon with ^{24}Na, and discovered that ^{24}Na was transported *out* of the cell against both concentration and electrical gradients. The efflux was blocked by metabolic (mitochondrial) inhibitors, e.g., cyanide, dinitrophenol, and also by the digitalis glycosides, oubain and strophanthidin. Oubain was effective only if applied inside the axon. Na^+ transport was also blocked by removal of K^+ from external media. K+ transport is coupled to Na+ transport. If Na+ is replaced by Li+, Li+ ions are not extruded. The extrusion of Na+ was thought to be due to an enzyme since injection of ATP could reinstate transport in DNP-poisoned axons.

In 1964 an enzyme, termed **Na/K ATPase** was isolated from crab nerve that displayed all these properties. The pump mechanism is still not completely understood. The enzyme is composed of two subunits - a catalytic subunit of about 100,000 daltons (a) and a glycoprotein subunit of about 45,000 daltons (b) whose function is unclear. Most of the a subunit consists of loops within the cytoplasm. There is a binding site for ATP, and one for sodium. In the presence of extracellular K, and extracellular binding site binds K which leads to dephosphorylation of the enzyme internally at the ATP binding site [This is the site of oubain action]. This causes a conformational change that transports K across the membrane. When Na binds to its internal site, the binding of ATP is enhanced, ATP is hydrolyzed, the enzyme is phosphorylated and the Na is transported back outside. The pump can operate at a reduced efficiency (~10%) in the absence of K^+.

In some cells, the transport ratio is 1:1 Na:K. However, in many cells, the transport ratio is 3:2 sodium:potassium which means that the pump is **electrogenic** in the hyperpolarizing direction. Stopping the pump causes a depolarization of the membrane. Because the ionic concentrations of Na+ and K+ are in steady state (constant), the leak ratio of Na and K must also be in the ratio of 3:2. The contribution of the pump to membrane potential can be calculated by:

$$Vm= (RT/zF)(r[K]_o+b[Na]_o/rK_i+b[Na]_i)$$

where r= pump ratio (3:2)
and b= *p*Na/*p*K
(This equation is derived in a similar way to the Nernst equation.)

The contribution of the pump to membrane potential is linked to the membrane potential and resting Na+ conductance. In squid, with a relatively low resting Na+ conductance, the pump adds about 6 mV of hyperpolarization to a resting membrane potential of -82 mV, or about 7%. In smaller mammalian cells with higher resting Na+ conductance, it could be as much as 15%.

Other Ion Pumps

There is also a Ca^{2+}-Mg^{2+} ATPase in neurons, similar to the Na^+-K^+ ATPase

Many cells also have an outwardly directed Cl- transporter. In most mature mammalian CNS neurons $[Cl^-]_i$ is regulated is handled by the neuron-specific potassium-chloride-cotransporter 2 (KCC2). The transporter is non-electrogenic - 1 Cl- ion and 1 K+ ion are transported in. Thus, the transporter function is linked to Na^+/K^+ ATPase activity and requires energy even though the transporter itself is not directly ATP-dependent. With KCC2 operating, the intracellular concentration of Cl- is maintained slightly lower than would be dictated passively by the membrane potential, and the result of an increase in Cl- conductance during a synaptic potential is hyperpolarizing. This is a major factor in the function of $GABA_A$ synaptic transmission. Interestingly, KCC2 is absent in most neonatal mammalian neurons causing $[Cl^-]_i$ to be higher than in adults, leading to depolarizing $GABA_A$ responses that are sometimes actually excitatory.

There is another Cl- extrusion mechanism in some neurons called the Na+- dependent anion exchanger (NDAE) that exchanges intracellular Cl^- and an H^+ ion for extracellular Na+ and $HCl0_3^-$ (bicarbonate).

In other cases (squid axon and vertebrate muscle), the Cl^- pump is inwardly directed in which case increasing Cl^- conductance causes a slight depolarization . *In either case, the contribution of Cl- to the resting potential is very slight,* on the order of 1 mV in a squid axon.

4. The Constant Field Equation

The Nernst equation can't be used to calculate the resting membrane potential since it applies to only one ion at a time, and a cell membrane is differentially permeable to several different ions. However, by a similar derivation, an equation can be constructed that describes the membrane potential as a function of the relative permeabilities and equilibrium potentials of all permeant species.

Start by expressing the flux of each ion as function of membrane voltage, concentration difference and *p.* Then convert flux (moles/cm^2/sec) to current by multiplying by the faraday constant.

$$\text{net flux} = \rho_k z V' \frac{K_o - K_i e^{zV'}}{e^{zV'} - 1}$$

so the corresponding currents for each ion are:

$$i_K = F\rho_k z V' \frac{K_o - K_i e^{zV'}}{e^{zV'} - 1}$$

$$i_{Na} = F\rho_k z V' \frac{Na_o - Na_i e^{zV'}}{e^{zV'} - 1}$$

$$i_{Cl} = F\rho_k z V' \frac{Cl_i - Cl_o e^{zV'}}{e^{zV'} - 1}$$

Since the membrane potential is not changing , the sum of all currents = 0
Factor out FV'($e^{v'}$-1) and rearrange.

$$p_k(K_o - K_i e^{V'}) + p_{Na}(Na_o - Na_i e^{V'}) + p_{Cl}(Cl_i - Cl_o e^{V'}) = 0$$

or

$$p_k K_o + p_{Na} Na_o + p_{Cl} Cl_i = e^{V'}[p_K K_i + p_{Na} Na_i + p_{Cl} Cl_o]$$

rearranging gives

$$e^{V'} = \frac{p_k K_o + p_{Na} Na_o + p_{Cl} Cl_i}{p_K K_i + p_{Na} Na_i + p_{Cl} Cl_o}$$

recalling that $V' = V_m F/RT$ and substituting we get

$$V_m = \frac{RT}{F} \ln \frac{p_k K_o + p_{Na} Na_o + p_{Cl} Cl_i}{p_K K_i + p_{Na} Na_i + p_{Cl} Cl_o}$$

This is the **constant field equation**, or **Goldman-Hodgkin-Katz equation** and represents the membrane potential at equilibrium for all permeant species, weighted by their respective permeabilities. If we assume that P_K:P_{Na}:P_{Cl} is 1:0.04:0.45 at rest, but

1:20:0.45 at the peak of an action potential, then from the numbers at the beginning of the lecture for squid axon ion concentrations we can calculate the resting membrane potential:

Em= 58log(1)(20)+(.04)(440)+(.45)(52)/(1)(400)+(.04)(50)+(.45)(560)/
= 58 log (20+17.6+23.4)/(400+2+252)
= 58 log 61/654
= 58 log .0932
=-59.7 mV

[I leave it to you to use the Goldman-Hodgkin-Katz equation to calculate what the membrane potential would be at the peak of the action potential. It's not strictly kosher to do this, since the membrane potential is rapidly changing during an action potential and one assumption of the derivation is that the potential field is constant, (hence the name, constant field equation), but the answer that obtains is very close to the observed measurements.]

This is for squid axon. For mammalian CNS neurons the potassium gradient is higher, the relative sodium permeability a little lower so the resting membrane potential is lower

[In general, cells with low E_m have higher resting Na^+ permeabilities- e.g., retinal receptor cells have E_m around - 40 mV due to a sizable resting Na+ conductance. When they absorb a photon of light they hyperpolarize as the Na+ conductance is shut off. Red blood cells also have a very low resting membrane potential due to a high Na^+ conductance.]

Note that one can verify the insignificantly small nature of Cl- role in the resting potential that was claimed above by deleting it from the constant field equation and recalculating:

=58 log (20+17/400+2)
=58 log 37/402
=58 log
= -60.09 mV
vs. -59.7 mV with Cl^- included.

Summary of the critical points:

1. The movement of an ion across the membrane is governed by two forces: its concentration difference and the electrical potential difference across the membrane.

2. Membrane potentials are produced by only thing, namely an excess of anions (or cations, if the inside of the cell is happened to be positive with respect to the outside) in the cell, compared to the number of cations. The charge disequilibrium only occurs with $\pm$ 1 μm of the cell membrane and is intimately tied to the membrane capacitance.

3. Electrical forces are very much more powerful than diffusional forces produced by a concentration difference, which means that relatively few excess ions are needed to counter concentration differences.

4. Neuronal membranes at rest are not in equilibrium, due to the steady leak of K^+ and Na^+. They are in *dynamic equilibrium* or *steady state* and require energy input to the Na^+-K^+ pump to remain there. [It has been estimated that 50% of the metabolic energy

consumed by the mammalian brain goes towards the operation of the various pumps required to maintain membrane potential.]

III. Introduction to Electrotonus

Neuronal membranes support passive and active electrical signaling. Passive electrical potentials, termed electrotonic potentials, spread through neuronal membrane rather slowly, and do not require the participation of voltage or transmitter-dependent ion channels (which will be covered in subsequent lectures). The methods for analyzing the flow of current in neuronal membranes have their origins in the equations first formulated by Lord Kelvin (William Thomson) in the mid 1800's to calculate the attenuation of signals in transatlantic undersea telephone cables stretching from the U.S. to Great Britain. These partial differential equations are referred to as **cable equations**. In some ways, neurons may be considered like leaky undersea cables. They both have highly conductive fluids (sea water or extracellular fluid) surrounding an insulated conductor. Some of the solutions to the cable equations, in the time and space domains, are of special relevance to the passive spread of electrical potential in neurons. This passive change of potential with respect to time and/or distance is called **electrotonus**.

A. Time Constant (t)

If we inject some positive (or negative) charges into an axon or dendrite through an intracellular recording electrode (similar to the situation that obtains when a synaptic conductance increase takes place) we observe that the membrane potential does not charge (or discharge) immediately to its final value. Electrotonic potentials have slow onsets and offsets due to the membrane capacitance. The time required to charge the membrane potential to 1-1/e (63%) of its steady-state value is the **time constant**, and is given by:

$$t = R_m C_m$$

During the rising or falling phase of an electrotonic potential, the time dependence of the voltage change is given by one solution of the cable equations in time:

$$V_t = V_0(1-e^{-t/t})$$

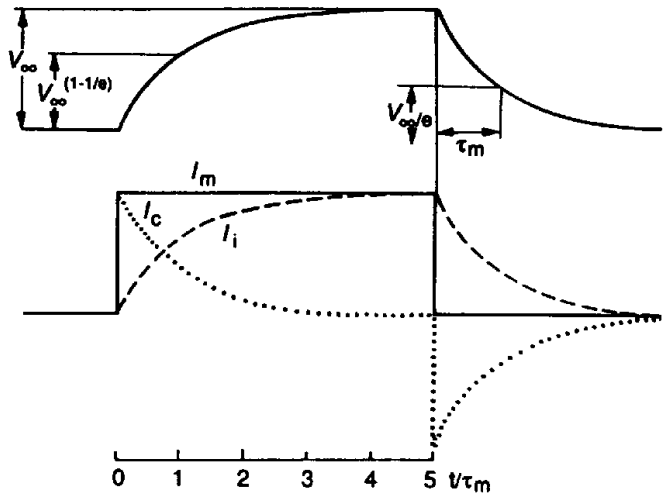
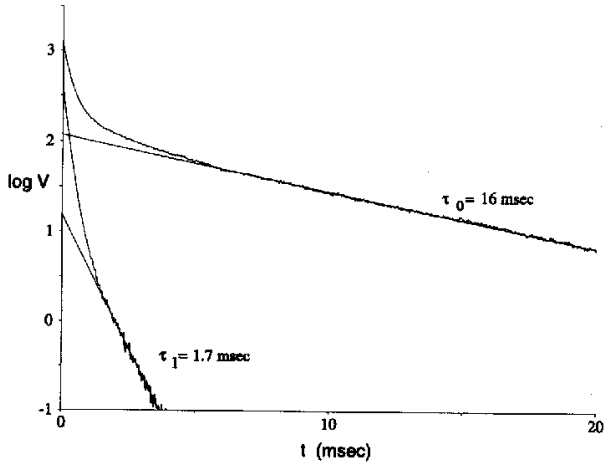


FIG. 13.10. Time course of charging and discharging of a passive membrane capacitance in parallel with a passive membrane resistance, in response to a current step. Abbreviations: I_m, applied membrane current: I_c, current through the capacitance; I_i, current through the ionic leak; V_∞, steady-state voltage, τ_m, membrane time constant. (Modified from Jack et al., 1975.)

A smaller t favors faster charging and discharging. t is an important variable in determining the **temporal summation** characteristics of a neuron. A large (slow) t increase the time over which inputs can temporally summate, whereas a smaller (faster) one decreases the minimum time intervals over which a neuron can discriminate individual inputs varying in time . [Note that because $t=R_mC_m$, and since C_m is very nearly constant for biological membranes, changes in t are good predictors of changes in R_m.]

In reality, the voltage response of any passive cable structure (e.g. a neuron and its dendrites) can be expressed as a sum of exponentials like the one above. The additional terms describe the voltage resulting from the flow of current into different parts of the cell and its dendrites. For neurons, the slowest time constant, t_0, is called the membrane time constant, t_m. The others are termed equalizing time constants and are faster. Time constants are measured by injecting square wave current pulses into neurons and observing the change in the voltage over time. The resulting curve is fit with the exponential function shown above
and t_0 is derived. If desired, the curve fit by t_0 can be subtracted from the original curve and the remaining curve fit with an exponential again, yielding t_1, the first equalizing time constant, and so on.



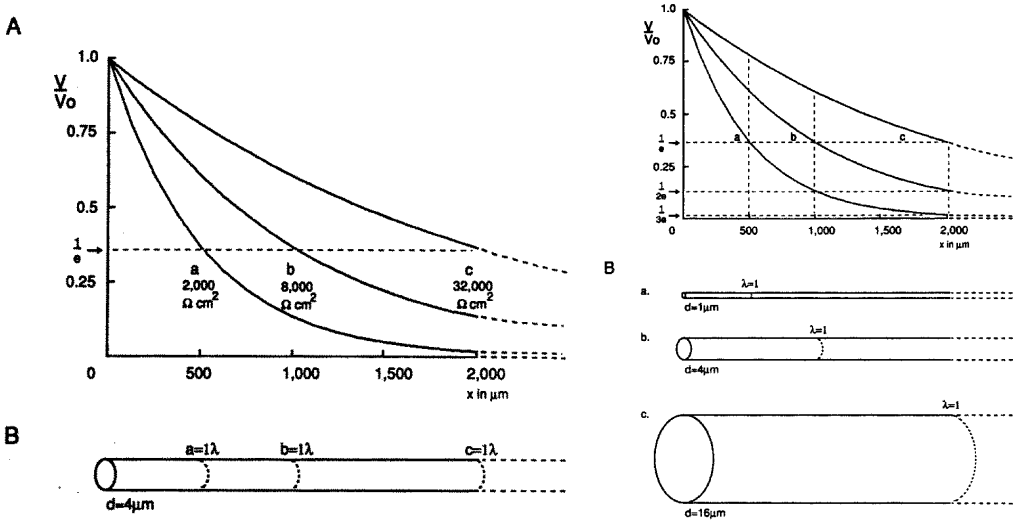
B. Length Constant (l)

If we inject some positive charges into an axon or dendrite, and record the voltage at varying distances from the injection site, we observe that electrotonic potentials decay over distance according to the ratio of R_m and R_i, and the diameter of the dendrite. The distance over which the potential decays to 1/e of its amplitude at the source is called the **length constant, l,** and is given by:

$$\lambda=\sqrt{r_m/r_a} \qquad \lambda=\sqrt{\frac{R_m}{R_i}}\cdot\frac{d}{4}$$

The voltage at distance x from the site of current injection (or the site of a synaptic conductance change) is given by another solution to the cable equations in space:

$$V_x=V_0e^{-x/l}$$



When x=l, $V_x = 0.37V_0$. This means that the length constant is the distance at which passive potentials decay to 1/e (37%) of their original value. [Do not be confused between the length constant, usually measured in μm, and the electrotonic length, which is expressed in units of l. A long length constant means that the cell inputs terminating over great distances of the neuron are electrotonically close while a long electrotonic length means just the opposite. Also, by convention, the *time* constant, t, is defined as the time required to rise to 63% (1-1/e) or decay to 37% (1/e) of its maximum value whereas the length constant, l, is defined as the distance over which a potential decays to 37% (1/e of its initial value.)]

For most dendrites in the CNS, the length constant is between 100 and 1000 μm. The length constant is an important variable in determining the spatial summation characteristics of a neuron as well as the speed of active action potential conduction. l is an important contributor to determining the extent of spatial summation by a neuron. The larger the l, the greater the surface area over which the neuron can summate inputs. **Note that myelination not only increases Rm/Ri, but also decreases C since the distance between the plates increases.**

[As we shall see in later lectures, the names time and space constant are something of a misnomer. In fact, since the resistance of the neuronal membrane is constantly subject to modification by synaptic input and voltage-dependent phenomena, perhaps it would have been better to call them time and space *variables* or *parameters*, rather than constants.]