PRINCIPLES OF ANIMAL PHYSIOLOGY

PART I.

1. PHYSIOCOCHEMICAL BASES OF CELLULAR EXCITABILITY.

- Ionic asymmetry: Donan-Gibbs equilibrium
- Equilibrium potential (or voltage): Nernst Equation.
- Membrane Potential (also called constant field equation). Goldman-Hodgkin-Katz.
- Balance maintenance: Na^+/K^+ exchange pump.

2. ACTION POTENTIAL

- Analysis of the electric signal: Resting potential, Threshold Voltage,
- Rising phase, Overshoot and
- Refractory phase.
- Na^+ Channel.
- K^+ Channel.

3. CONDUCTION OF ELECTRICAL IMPULSES.

- Speed of propagation of Action Potential Optimization patterns: giant axons and myelinated axons
- Saltatory Conduction in mielinated axons

4. TRANSMISSION OF INFORMATION BETWEEN NEURONS.

- Synaptic graduated potentials.
- Electrical vs. chemical synapses.
- The chemical synapses: Neuromuscular junction as a tool to reveal synaptic currents. Presynaptic events: Quantic release of neurotransmitters. Postsynaptic events. Neurotransmitters synthesis: slow vs. fast transmission.
- Synaptic potentials: Excitation & Inhibition. The reversal potential.

5. INTEGRATION AT SYNAPSES

- Summation: spatial and temporal.
- Synaptic plasticity: Homosynaptic modulation. Facilitation. Depression. Short term and long term.

TERMS AND CONCEPTS I: MEMBRANE PROPERTIES

1. DIFFUSION

Suspended or dissolved molecules move randomly dispersing form regions of higher concentration to regions of lower concentration. This thermally influenced process is called diffusion. The FICK EQUATION can define the rate of diffusion of a solute:

$$dQ_s/dt = D_s\, A\, (dC_s/dx)$$

in which dQ_s/dt is the quantity of s diffusing per unit time (rate of diffusion), D is the diffusion coefficient for s, A is the cross-sectional area and dC_s/dx the change of concentration of s with distance (concentration gradient)

2. MEMBRANE FLUX

If a given permeable solute is present at both sides of a membrane it exhibits a unidirectional flux in each direction. Both fluxes are considered to proceed independently and when influx equals efflux (in the case of a cell) the net flux is zero.

$$J = dQ_s/dt \quad (M\ cm^{-2}\ s^{-1})$$

3. MEMBRANE PERMEABILITY

The rate at which a solute passively crosses the membrane under a specified set of conditions is called permeability. Assuming that the membrane is a homogeneous barrier and that a continuous concentration gradient is kept then

$$dQ_s/dt = P\,(C_1 - C_2)$$

in which C_1 and C_2 are the concentrations at both sides of the membrane and P is the permeability constant (v = cm/s). ***This expression excludes electrolytes*** *since their flux is also dependent on electrical gradient.*

Plasma membranes are lipid bilayers and though their permeability to water is much higher than predicted from the oil/water partition coefficient, maximum rates at which ions can cross them is less than 10^{-8} when compared with diffusion through water so that they can be considered negligible.

4. OSMOSIS

The movement of water across a membrane down its concentration gradient is called osmotic flow. In an experimental set-up in which a semi permeable membrane separates distilled water from a compartment in which a non-permeable solute is present, a net transfer of water from the compartment in which concentration is 0 occurs. Water increase ceases when hydrostatic pressure generated at the concentrated compartment equals osmotic pressure (π).

$$\pi = RTC = nRT/V$$

where R is the molar gas constant (0.082 L x atm x K-1 x mol-1), T is absolute temperature, C is concentration (moles of solute per litre of solvent or osmolarity), n is the number of mole equivalents of solute and V is the volume in litres.

5. OSMOLARITY AND TONICITY

When different solutions that have equal amount of dissolved particles per unit volume are put in contact through ideal (only water is allowed through) semi permeable membranes no net flux of water occurs since osmotic pressure exerted is the same. Such condition is called isosmotic. The term ***tonicity*** *refers to cell response to immersion into a particular solution: if water is gained the solution is called* ***hypotonic*** *and* ***hypertonic*** *if it causes dehydration. If cells or tissues worked as perfect osmometers, osmolarity and tonicity would be similar which is not generally the rule. Hypotonicity depends directly on the rate of cellular intake of the solute since water flow follows solute flow. For example: two solutions isosmotic to seawater elaborated with NaCl and, alternatively with $CaCl_2$ result in fact isotonic and hypotonic respectively to sea urchin eggs (isosmotic = isotonic while in sea water).* ***(Check for comparative permeability and intracellular concentrations of Na, Cl and Ca to propose an explanation).***

6. THE DONAN EQUILIBRIUM.

When a semi permeable membrane that totally excludes permeation to one of the ionic substances separates two electrolytic solutions, freely permeable solutes distribute asymmetrically between the two compartments.

TERMS AND CONCEPTS I: MEMBRANE PROPERTIES

7. THE ELECTROCHEMICAL POTENTIAL

When two electrolytic solutions are put in contact through a semi permeable membrane, both chemical gradient and electric field have an effect on passive diffusion. For a given ion, the sum of both forces determines the net electrochemical gradient.

8. MEMBRANE RESSISTANCE AND CONDUCTANCE

The resistance of a membrane (R) is a measure of its impermeability to ions and equals to the inverse of conductance (G), which represents its permeability (R = 1/G). The Ohm's law describes the relationship between current, resistance and voltage across a membrane.

$$\Delta Vm = \Delta I \times R$$

in which ΔVm (volts) is voltage change across membrane, ΔI (amperes) is the current across membrane and R (Ω= ohms) is the electrical resistance of the membrane.
In an animal cell membrane channels increase conductance by allowing ions to diffuse. In fact ion channels increase both conductance and permeability given that molecules have both mass and electrical properties.

9. MEMBRANE CAPACITANCE

In an electric circuit a capacitor displays the property to separate electric charges when a voltage is applied: positive charges (cations) move towards the cathode and anions (negative charges) towards the anode. The amount of charges that can be separated depend on the thickness and the dielectric constant. The plasma membrane behaves as a capacitor maintaining a thin layer of exclusively negative charges in the inner side and an equal number of cations in the outer side. Bulk solutions on either side of the membrane remain electroneutral.
Capacitance of plasma membranes can be calculated considering a thickness of about 5 μm for the lipid bilayer and the dielectric constant for an 18-carbon-fatty-acid (≈3) giving a result of 1μF per square cm which adjusts well to actual measurements of capacitance in biological membranes.

10. THE NERNST EQUATION

This equation allows calculation of equilibrium potential for single ions. In this formulation it is resolved as the electrical force that equals in magnitude and differs in sign to the osmotic force created by the chemical gradient, provided that the ion is permeable.

11. THE GOLDMAN-HODKING-KATZ EQUATION

This formulation allows calculation of the dynamic steady-state potential in multiple ion systems. It is basically an extension of the Nernst equation to include concentration gradients for every ion modified by the particular permeability constants included as factors. Dynamic steady-state is an alternative to the term "constant" which applies better to biological systems in which continuous exchanges of matter an energy are taking place.

12. THE MEMBRANE POTENTIAL & THE RESTING POTENTIAL

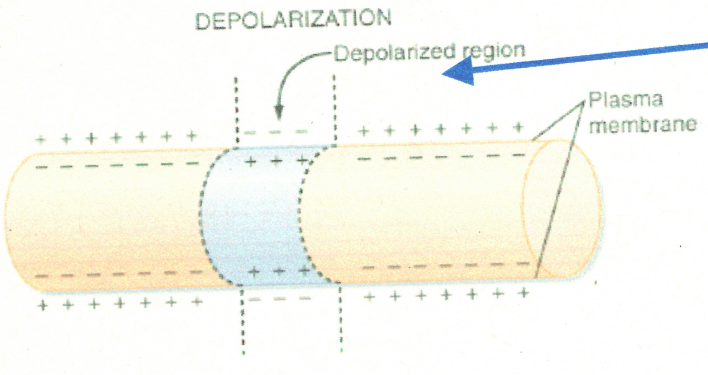
Since physicochemical characteristics of the animal cell membrane and electrochemical gradients among intra and extra cellular fluids are similar in any animal cell, dynamic steady state potential, that is the membrane potential taken as the electrical force operating across the membrane differs little among cells. The fact that intracellular fluid largely exceeds in potassium to extra cellular liquid along with highest permeability for this ion (P_k = 1) result in an electrical force that closely approaches to equilibrium potential for K^+. Real recordings as well as Goldman equation predictions indicate that membrane potential is slightly hypo-polarized as regards to equilibrium potential for K^+. This condition results from the co-participation of sodium (concentration asymmetry between intra and extra cellular fluids closely mirrors potassium distribution) that thought scarcely permeable when compared to potassium ($\approx 10^{-2}$) is submitted to a large electrochemical gradient.

Two particular types of cells, neurons and muscular cells, exhibit the so-called excitable membranes, the term excitable implying electrically excitable. In these cells, membrane potential can be altered by controlled active mechanisms to produce electrical signals: action potentials, synaptic potentials and receptor potentials. When these cells are activated to change their membrane potential they are said to be in "active state". By counter position the membrane potential exhibited when inactive (similar to any other cell) is termed the "resting potential".

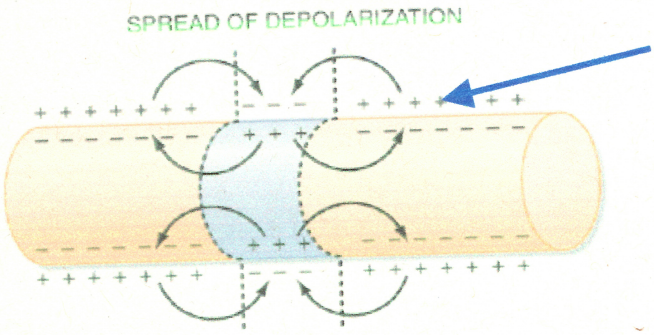
GENERATION AND CONDUCTION OF ACTION POTENTIALS

1. ANALYSIS OF THE ELECTRIC SIGNAL

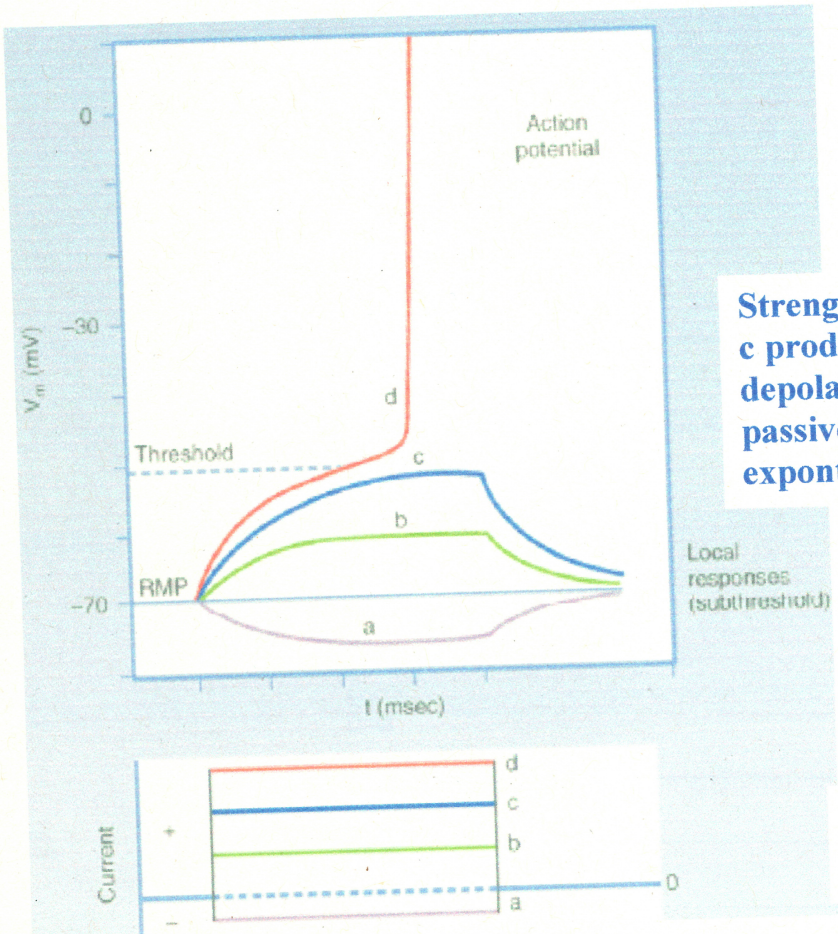
1.1. The Passive response: From Resting Potential to Threshold Voltage.



Initial Depolarization



Local circuit of Current flow



Strenght of Currents b and c produces sub-threshold depolarization that spreads passively and vanish expontaneously.

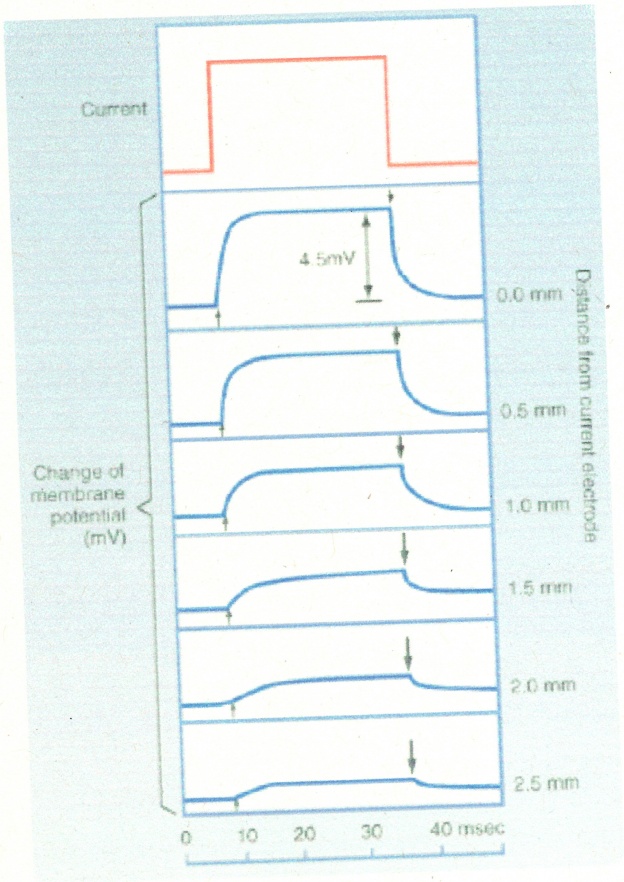
Principles of Animal Physiolog
Dr.M.Mercedes Ortega

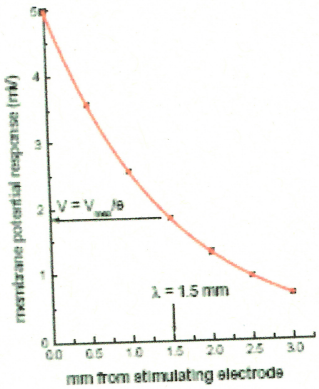
GENERATION AND CONDUCTION OF ACTION POTENTIAL

1.ANALYSIS OF THE ELECTRICAL SIGNAL

1.1. The Passive Response: The Lenght constant (λ)

The length constant (λ) is a mathematical constant used to quantify the distance that a graded electric potential will travel along an axon via passive electrical conduction. The greater the value of the length constant, the further the potential will travel.



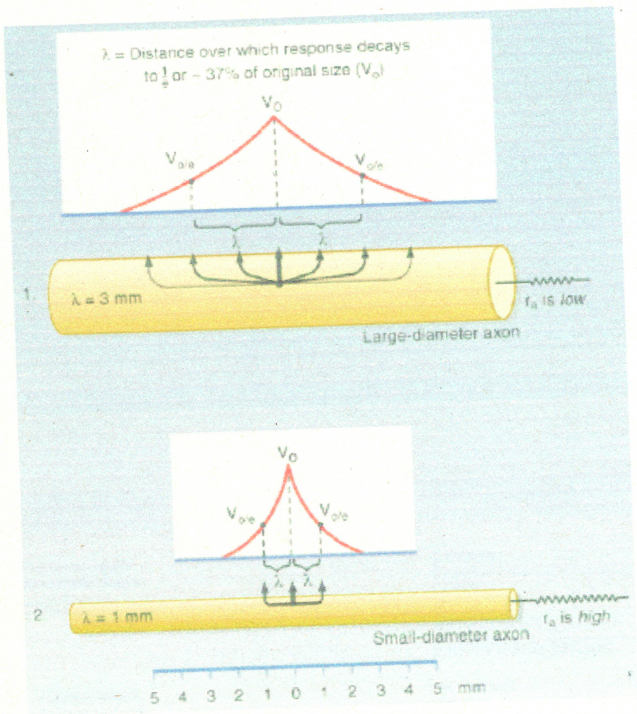


$$V_x = V_0 \, e^{-x/\lambda}$$

For $x = \lambda$

$$V_x = V_0 \, e^{-1} = V_0 \, 1/e = 0.37 \, V_0$$

V = voltage in mV



The length constant can be defined as

$$\lambda = \sqrt{R_m / (R_i + R_o)}$$

R_m = Membrane resistance

R_i = axoplasm resistance (in parallel to the membrane)

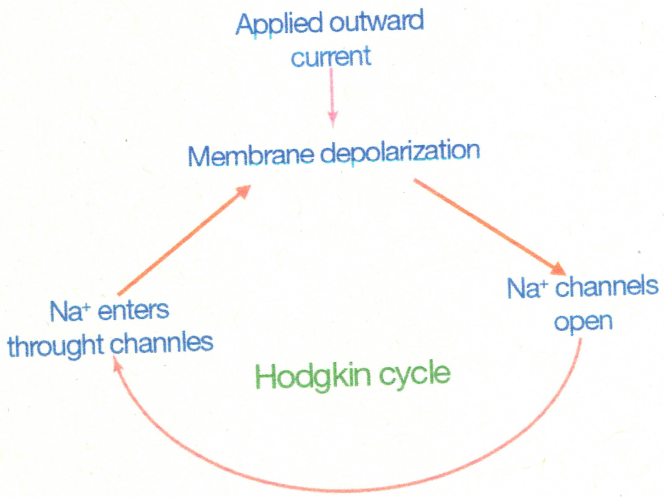
R_o = extracellular resistance which can be taken as negligible

GENERATION AND CONDUCTION OF ACTION POTENTIALS

1. ANALYSIS OF THE ELECTRICAL SIGNAL

1.2.The active response: from passive and graded depolarization to the spike: The Hodgkin cycle.

The Hodgkin cycle represents a positive feedback loop in which an initial membrane depolarization leads to uncontrolled deflection of the membrane potential to near VNa. The initial depolarization must reach or surpass threshold in order to activate voltage-gated Na+ channels. Opening of Na+ channels allows Na+ inflow which, in turn, contribute to recruiting more sodium channels and further depolarization of the membrane. This Na+ influx is brought about both by the Na+ concentration gradient, and the inside negative membrane potencial which create a strong electrical driving force for sodium ions. This cycle leads to a very rapid rise in Na+ conductance (gNa), which moves the membrane potential close to VNa. Conductance can be thought of as the electrical counterpart of permeability (p). Both conductance and permeability are proportional to the total number of open channels in the membrane.



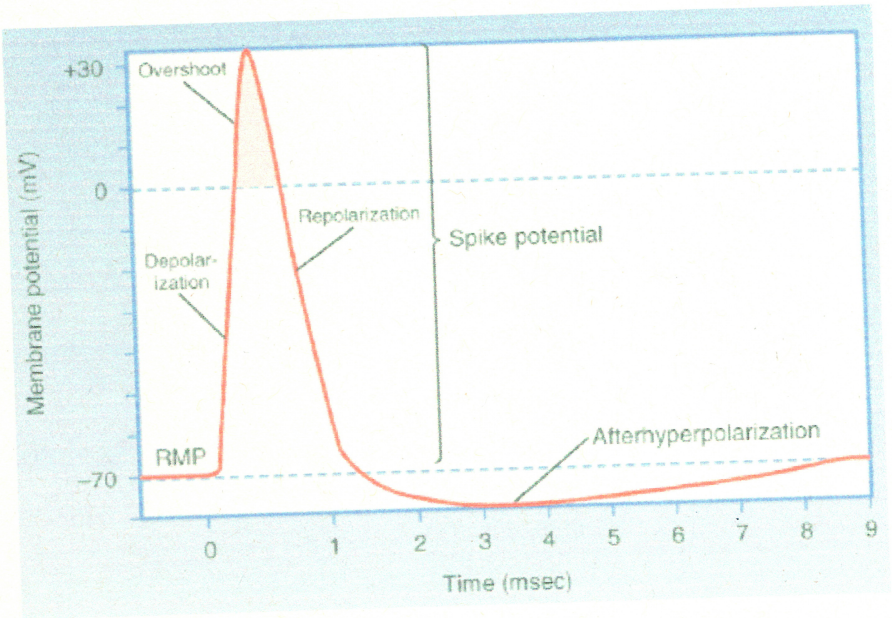
At the peak of the action potential, the membrane potential is close to VNa, but it never reaches VNa. There are two reasons for this.

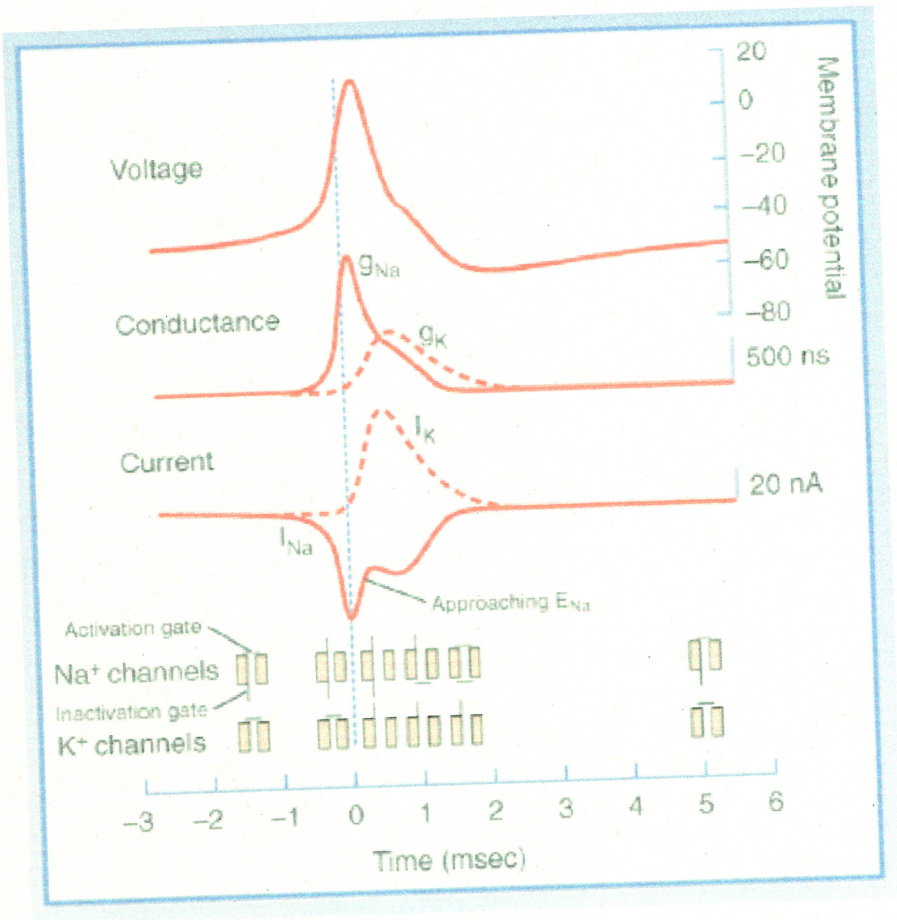
1. The voltage-gated Na+ channels begin to inactivate spontaneously very rapidly after opening. Channel inactivation "plugs" the pore of the channel so that Na+ ions can no longer pass through the channel permeation pathway. A cytosolic region of the Na+ channel actually blocks the Na+ permeation pathway of the channel. This has been referred to as the ball-and-chain model of inactivation. Ball refers to a globular cytoplasmic portion of channel protein that is tethered to the rest of the protein by a linker (or chain) part.

2. Neurons also have voltage-gated K+ channels that become activated by membrane depolarization (also at around the threshold voltage of -40 to -50 mV). Activation of the voltage-gated K+ channels, however, is much slower than that of voltage-gated Na+ channels and they are referred to as delayed rectifiers. Therefore, at the peak of the action potential, pK is greater than its value when the neuron is at rest, and movement of K+ out of the cell opposes the depolarization caused by the movement of Na+ into the cell

GENERATION AND CONDUCTION OF ACTION POTENTIALS

1. **ANALYSIS OF THE ELECTRICAL SIGNAL**
2. **The Active Response: Over threshold stimulus leads to a rapid depolarization based on transient but strong increase of conductivity for sodium ions (gNa) followed by a quick repolarization mediated by equally transient increase in conductivity for potassium ions (gK) resulting in a wave known as "spike".**



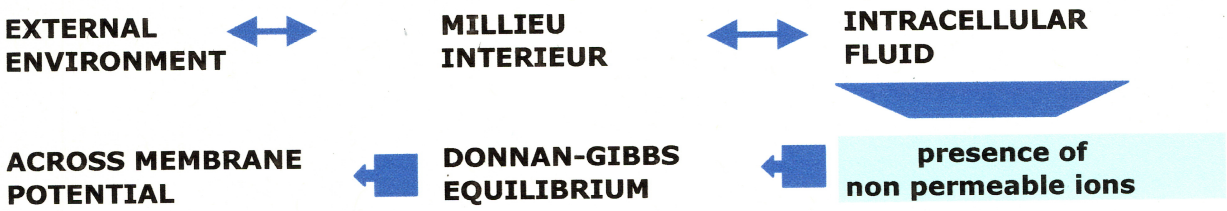


Class Group. Session 1.

1.Resting neuron
$[K^+]e$ = 10mM, $[K^+]i$ = 150 mM, $[Na^+]e$ = 120 mM y $[Na^+]i$ = 5 mM.
Membrane permeability for potassium is 150 time higher. Calculate membrane potential and equilibrium potential for both ions.

2.- Case study: A neuron shows at resting stage the following concentrations in Na^+ and K^+ for extracellular and intracellular liquids: $[K^+]e$ = 5mM, $[K^+]i$ = 120 mM, $[Na^+]e$ = 100 mM y $[Na^+]i$ = 10 mM. Knowing permeability for K^+ is 100 times higher than permeability for Na^+, membrane potencial for those conditions would be -75.4 mV. What would happen if intracellular K^+ concentration should be halved? And what about internal Na^+ concentration becoming double? Which modification would exert higher influence? Why? What would be maximum expected value for the action potential before and after altering Na^+ concentration?

OSMOTIC RELATIONSHIPS BETWEEEN COMPARTMENTS



UNITS IN mOsml/l

	Na+	K+	Ca++	Mg ++	Cl -	+	−
SQUID							
SEA WATER	470	10	20	50	550	550	550
BLOOD	450	20	20	50	570	540	570
AXOPLASM	50	400	0,3x10-3	10	100	460	100
							— 360

MEMBRANE POTENTIAL Vm = -60 TO -70 mV

298 mg/100 gr muscle (84% water) 354,76 mg/100 mg muscle water

	Na+	K+	Ca++	Mg ++	Cl -	+	−
FROG							
RIVER Water	0,5	0,05	0,8		1,35	1,35	0,85
BLOOD	112	1,9	2,2		116,1	116,1	116,1
MUSCLE	9,2	140	<10-3		3,5	149,2	3,5
							—145,7

Vm = -90 mV (FAA) − 140 molar eq.

	NA+	K+	Ca++	Mg ++	Cl -	+	−
MARINE CRAB							
SEA WATER	470	10	20	50	550	550	550
BLOOD	510	12		8	540	530	540
LEG NERVE	52	410			26	462	26
							— 436

Vm = -60 to -70 mV (FAA) − 422 molar eq. for *Callinectes*

	Na+	K+	Ca++	Mg ++	Cl -	+	−
MAN							
BLOOD	142	4	5,2	2	110	153	110
MUSCLE	10	156	3	26	4	195	4
							— 191

Vm = -75 mV Intracellular organic solutes = 180