UNIVERSITÀ
DI TORINO

Department of
Life Sciences
and Systems Biology

Cellular and Molecular Biophysics

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Corso di laurea in LM Biotecnologie Industriali- LM Fisica

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Electrical properties of cell membranes

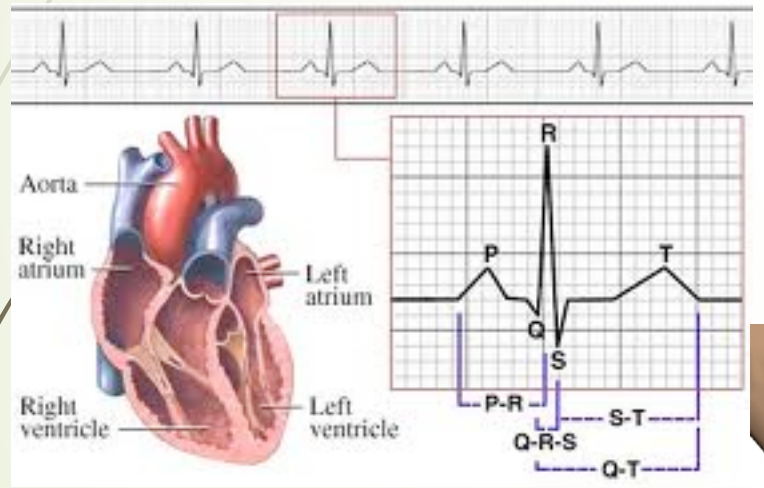
Cell excitability.

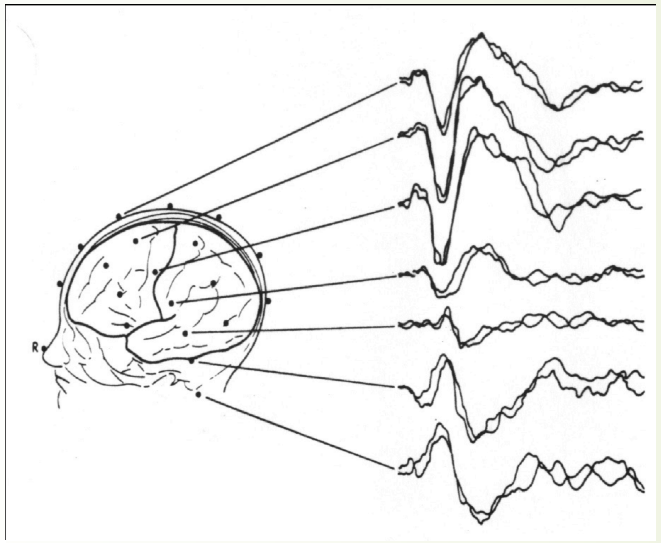
Action potential.

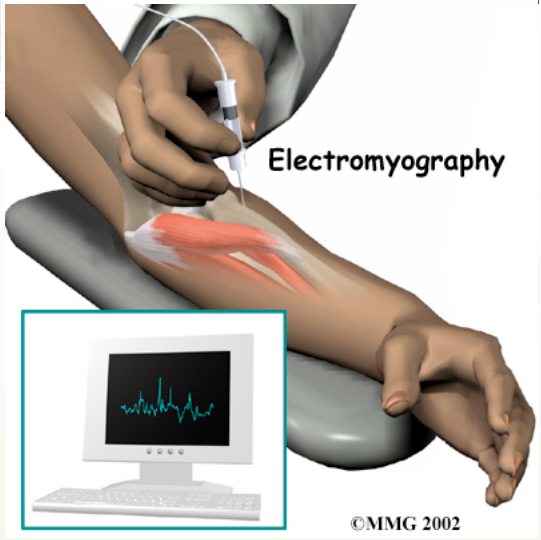
Membrane currents measurements by patch clamp technique

Measure of electrical signal from the entire body …

E.E.G.: electroencephalograms
ECG, EMG

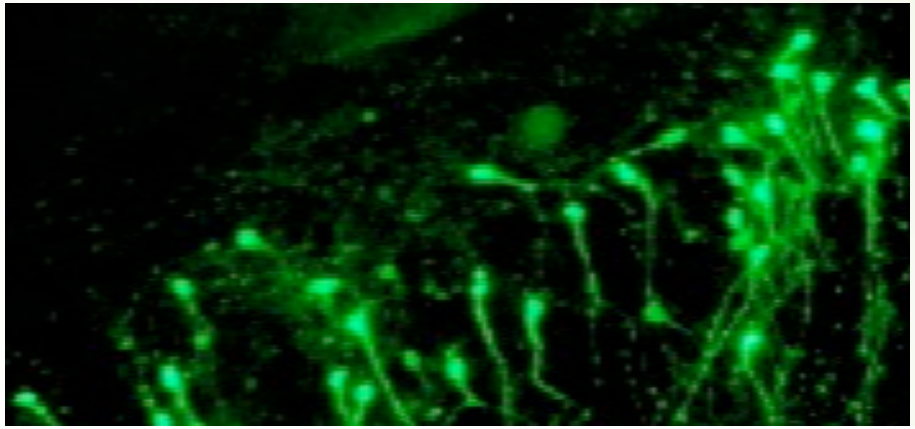


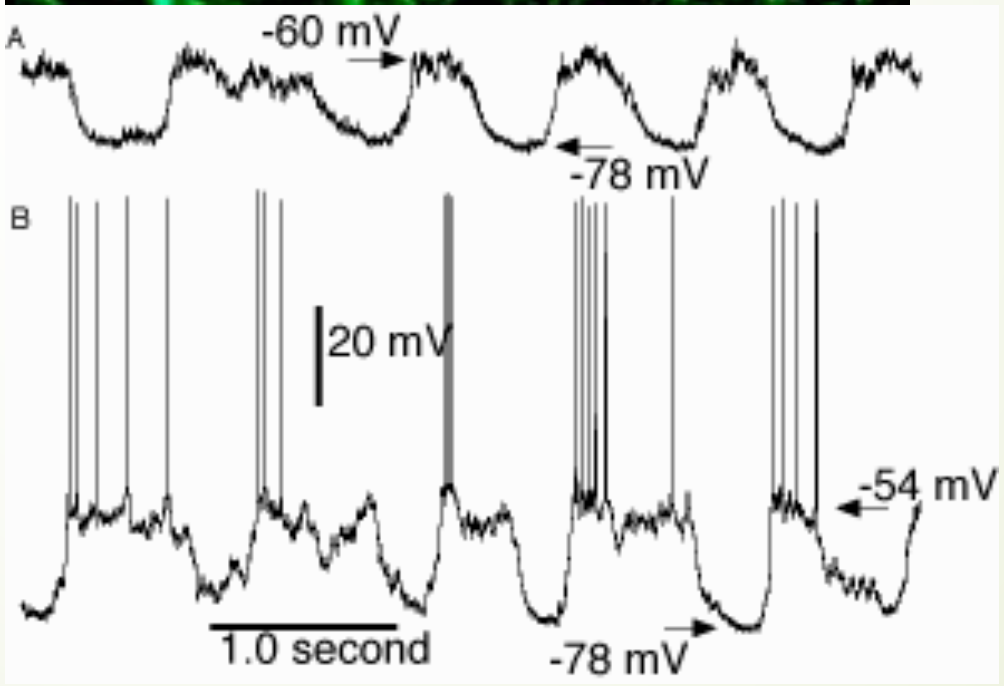




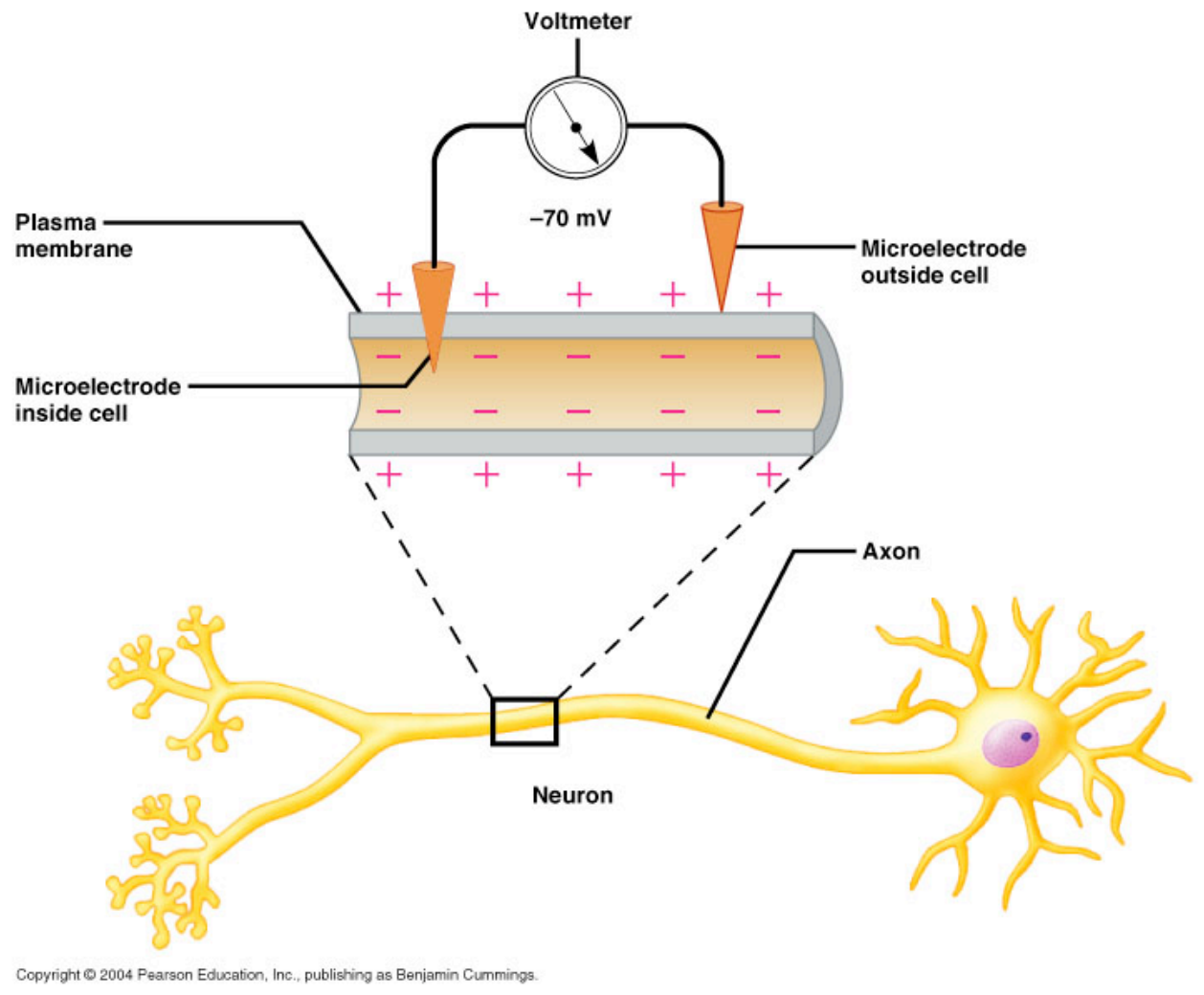
…to the cells in colture

Neurons in Culture

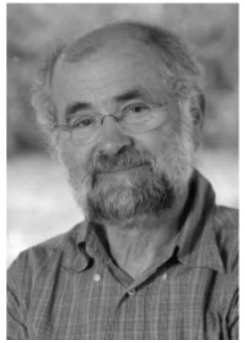




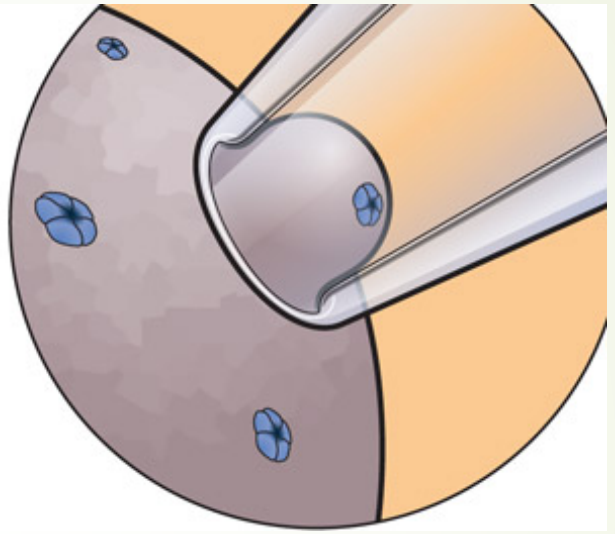
…to isolated cells



PATCH CLAMP technique

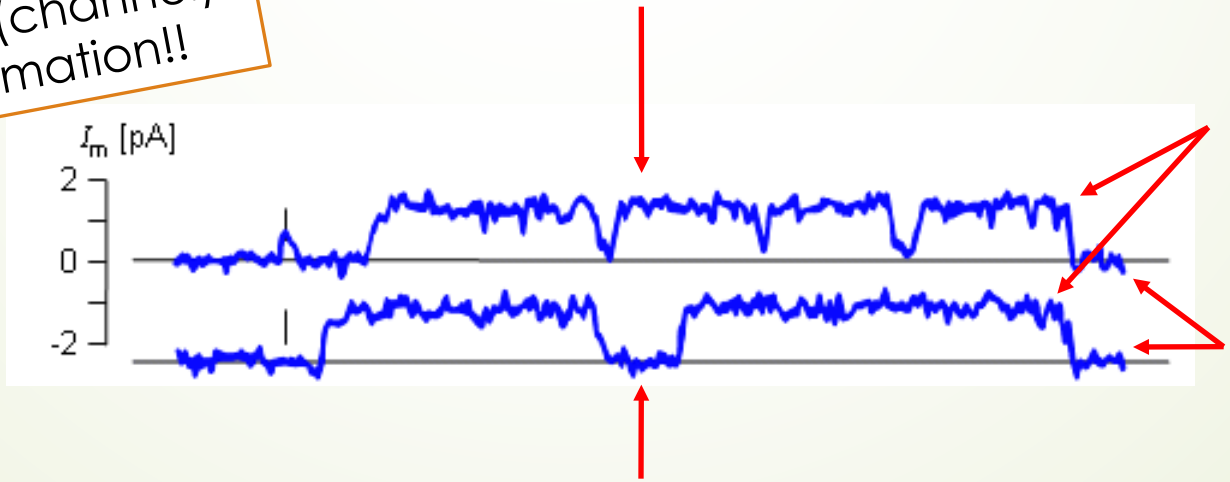


•**Erwin Neher and Bert Sakmann** developed the patch clamp in the late 1970s and early 1980s. They **received the Nobel Prize in Physiology or Medicine in 1991 for this work.**



Measurement of ionic currents flowing through the entire plasma membrane of a cell or a SINGLE CHANNEL:
high resistance seal

A single protein (channel) changes conformation!!



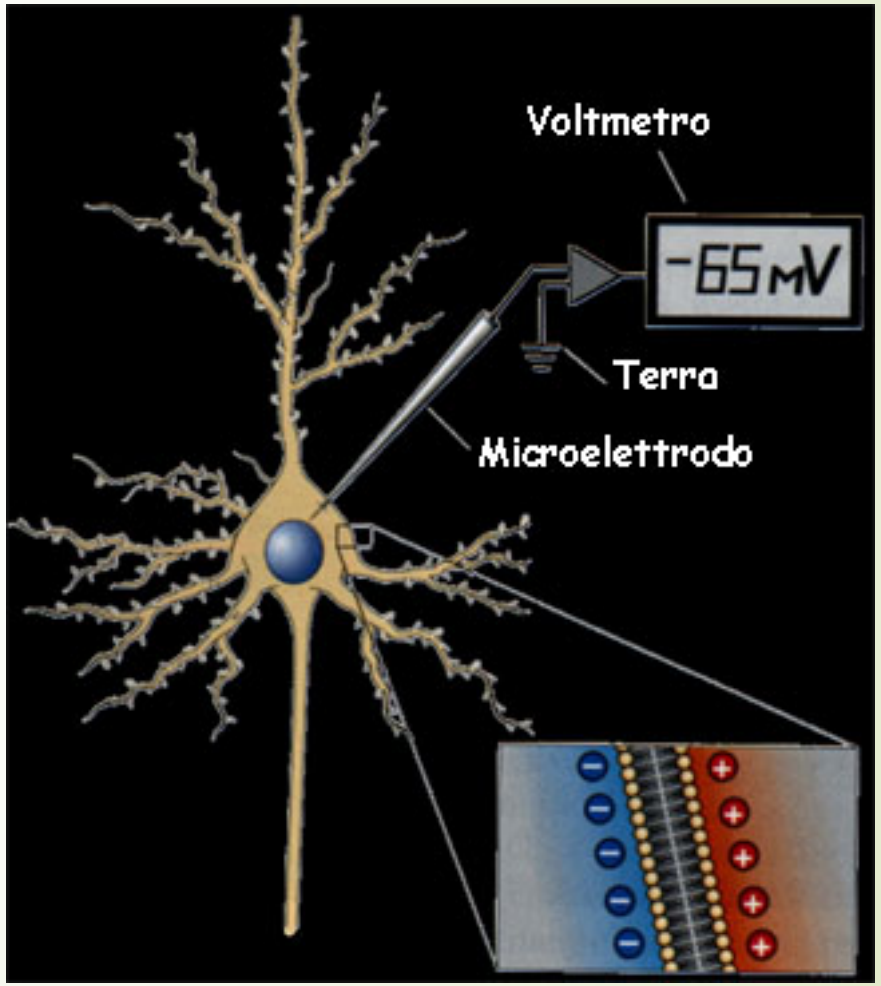
Open state

Closed state

MEMBRANE POTENTIAL

All cells present a **different charge distribution** at membrane sides. This generates a **MEMBRANE POTENTIAL DIFFERENCE** between intracellluar and extracellular sides.

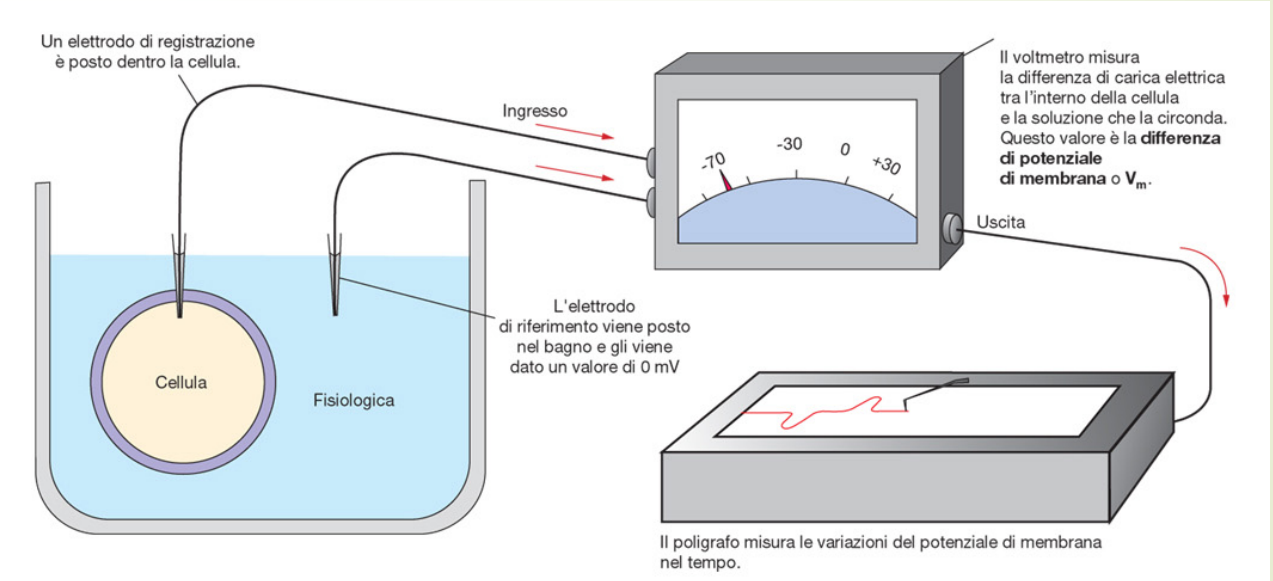
- The electric charges are due to te preence of ions in in the interstitial liquids
- **CATIONs** = ions + (K+, Na+, Ca2+….)
- **ANIONS** = ion - (Cl-….)



Experimental measure of the Membrane Potential (V_m)

A voltmeter measure the potential difference between intracellular and extracellular compartments. If we set to 0mV the extracellular compartment, we will measure a negative potential inside the cells

- In the majority of the cells the intracellular compartment is about 70mV negative as compared with the extracellular solution
- **V_m= -70mV**



V_m is determined by a different concentration of **K+ and Na+.**
This different concentration together with the concentrations of other ions such as **Ca2+** and **Cl-**, give rise to the accumulation of positive charges at the outer face of the membrane and a negative charges accumulation on the inner face, with a difference of about -70mV

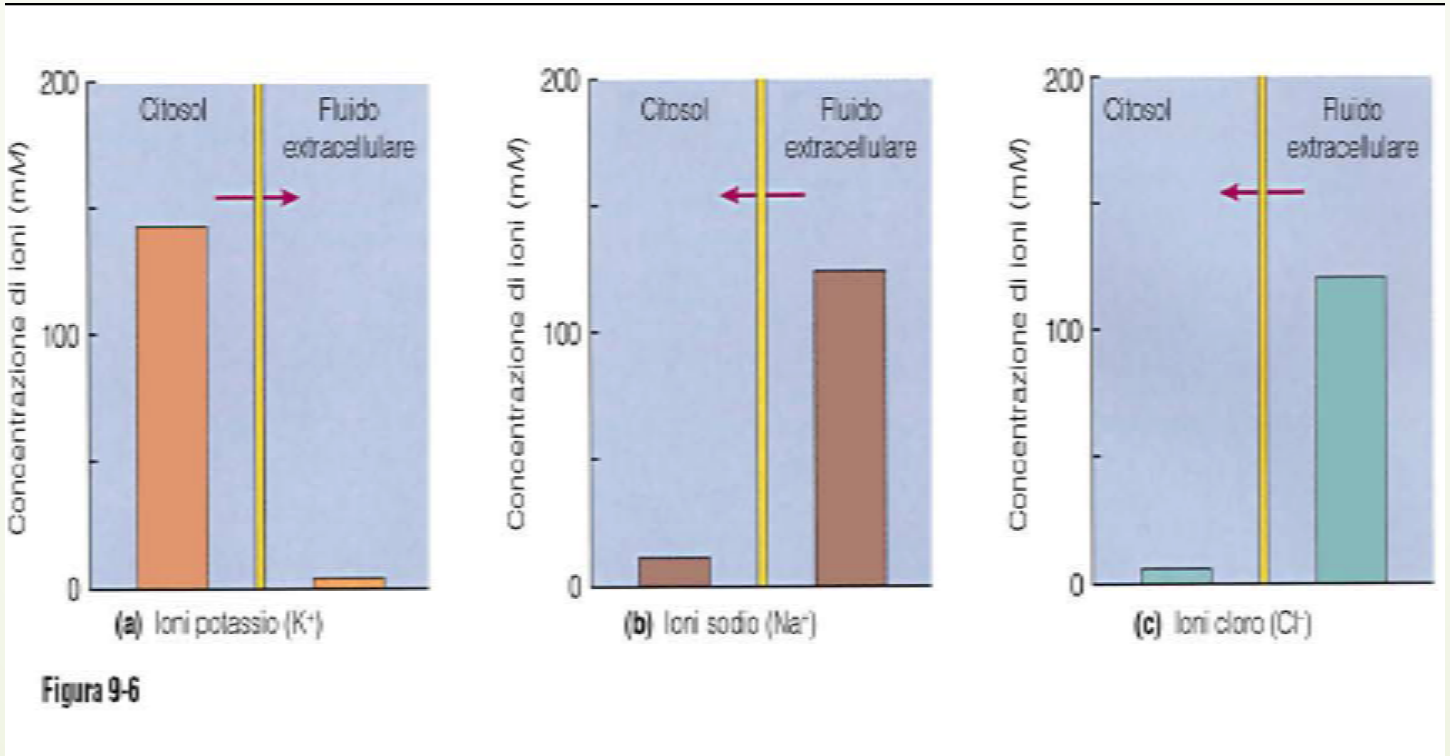
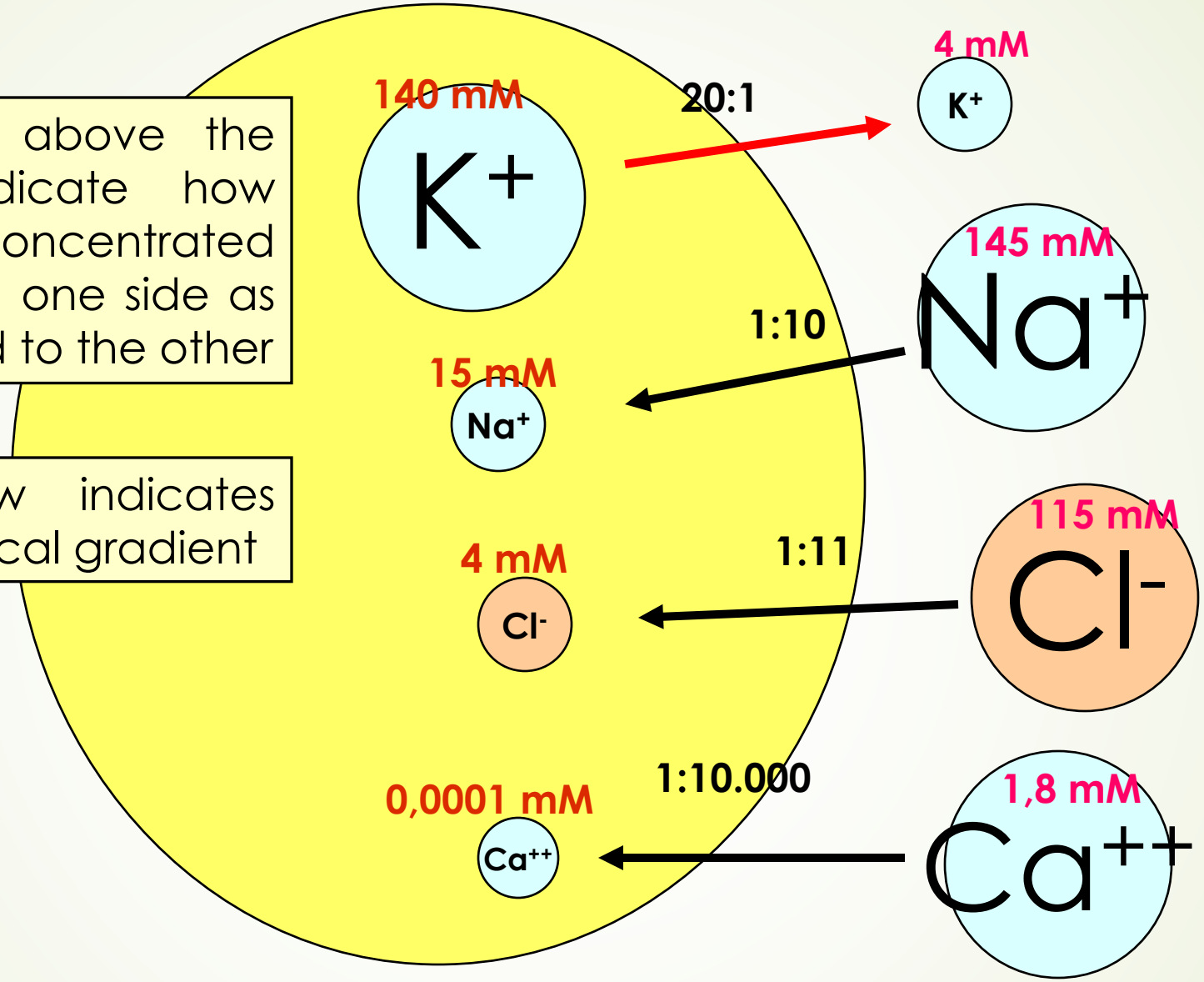


Figura 9-6

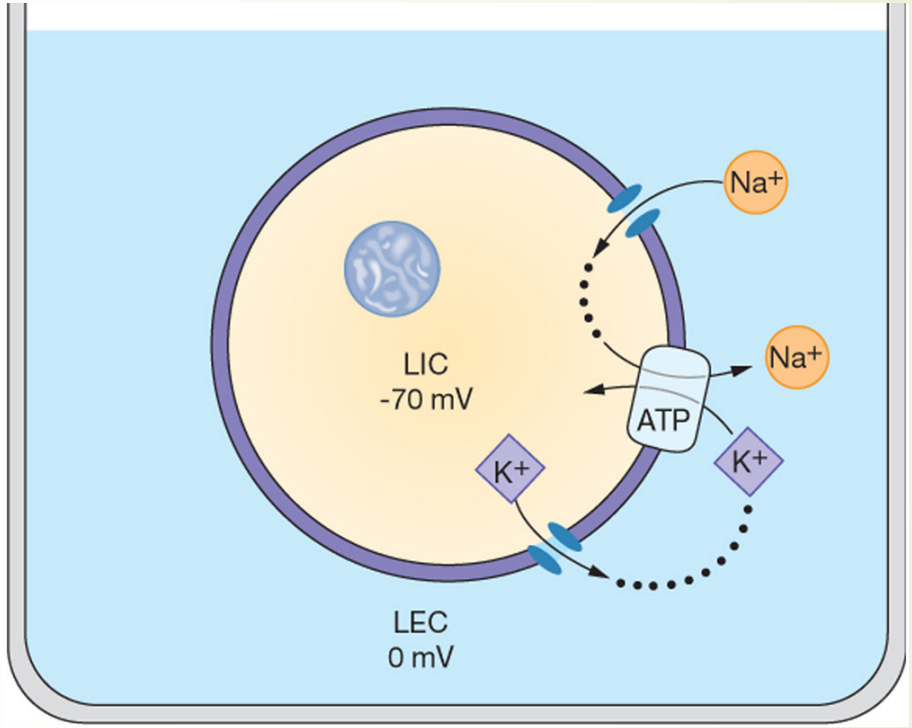
The ratio above the arrow indicate how much is concentrated the ion on one side as compared to the other

The arrow indicates the chemical gradient



Why the Vm in resting conditions is around -70mV?

Resting cells are permeable to **Na+ and K+.** As an average the cells are about **40 times more permeable to K+ than to Na+** (more K+ channels are open = leak channels) Vm is therefore closer to V_{eq}k+ = - 90 rather than V_{eq}Na+ = +60mV. Small amount of Na+ flow in the cells (leak channels) so that the Vm is less negative than if all Na+ was not moving



Na+/K+ ATPase maintains the gradients of Na+ and K+

Cell permeability to any ion changes with opening/closing of ion channels

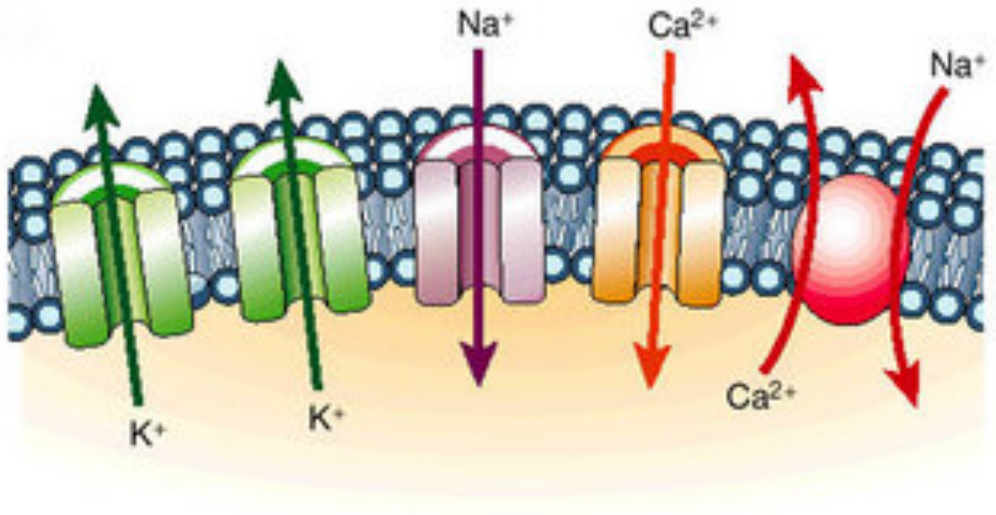
Direction of movement of one ion is dictated by the electrochemical driving force:

(V_m - V_{ion})

Resting Membrane potential

Nernst potential

Driving force



$$V_m = \frac{RT}{F} \ln \frac{P_K[K^+]_e + P_{Na}[Na^+]_e + P_{Cl}[Cl^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_e}$$

NERNST equation

$$V_{eq} = \frac{RT}{zF} \log \frac{C_{out}}{C_{in}}$$

NERNST equation

$$V_{eq} = \frac{RT}{zF} \log \frac{C_{out}}{C_{in}}$$

Equilibrium potential (E) for important ions in a neuron.

E_{Cl^-}	-70mV
E_{K^+}	-90mV
E_{Na^+}	+60mV
$E_{Ca^{2+}}$	+130mV

DF = $V_{membrane} - V_{eq}$ (V Nerst)

Direction of the flux:

NEGATIVE: influx of the ion

POSITIVE: efflux of the ion

Opposite direction for z-

Table I. Sign of electrochemical driving force (V_{DF}) and direction of ion flow

Ionic species	Sign of driving force ($V_{DF} = V_m - V_{eq.}$)	Direction of ion flow
Cation	+	Outward
	0	No net flow
	−	Inward
Anion	+	Inward
	0	No net flow
	−	Outward

+ refers to $V_{DF} > 0$, − refers to $V_{DF} < 0$, and *0* refers to $V_{DF} = 0$. When $V_{DF} = 0$, the ion is in electrochemical equilibrium. *Cation*, positively-charged ion; *Anion*, negatively-charged ion; *Outward*, ion movement out of the cell; *Inward*, ion movement into the cell.

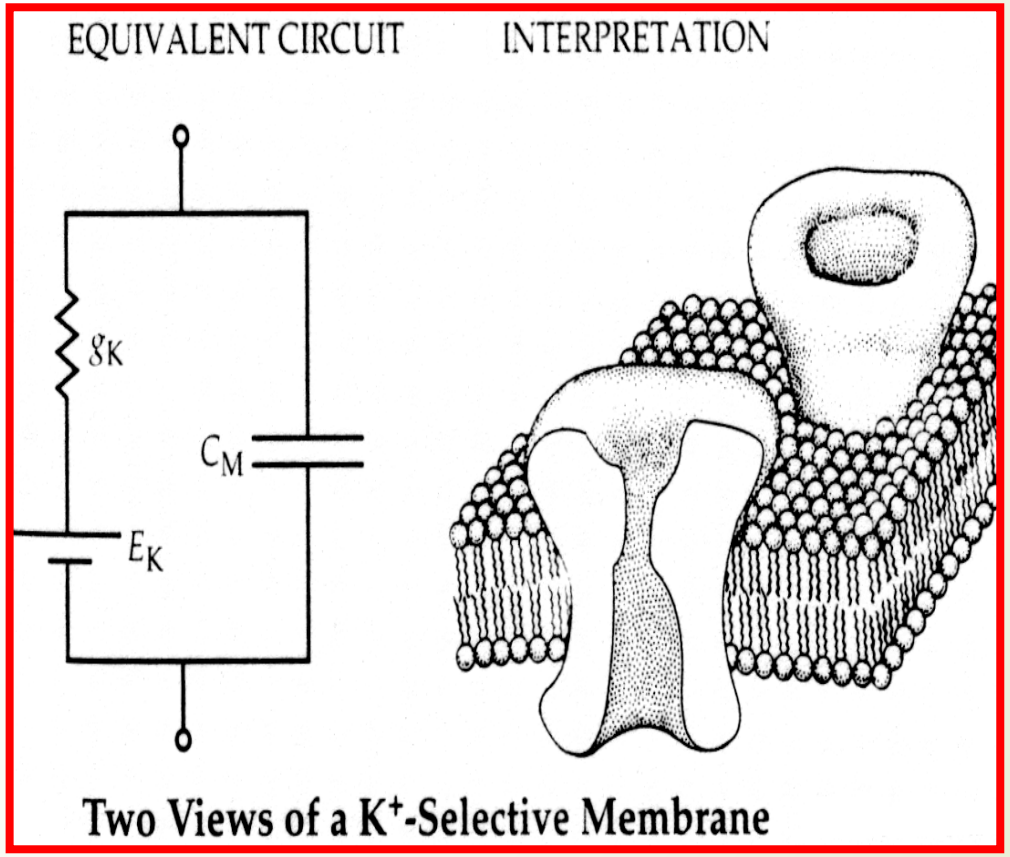
Electric representation of the cell membrane

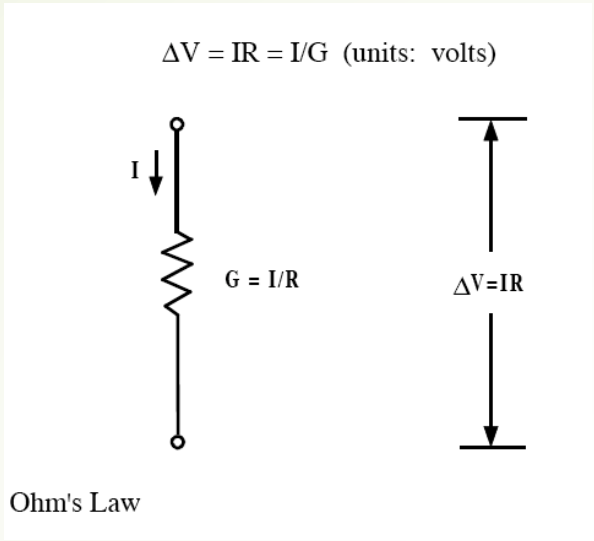
A membrane behaves electrically like a

ohmic conductance

in parallel with a

capacitance





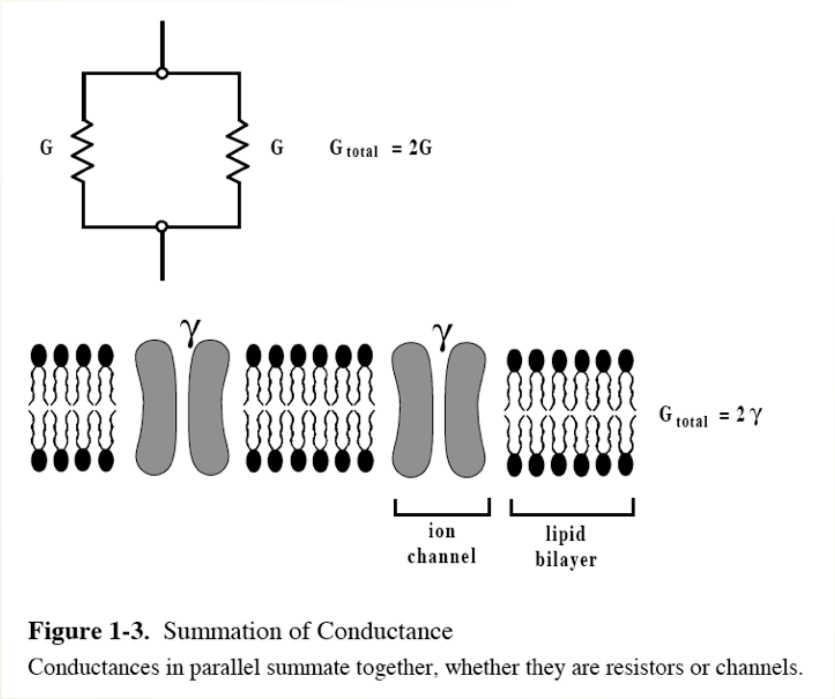


Figure 1-3. Summation of Conductance
Conductances in parallel summate together, whether they are resistors or channels.

OHM's law

$$I=\frac{\Delta V}{R}$$

$R =$ resistance

$$G=\frac{1}{R}= \text{ conductance}$$

$$I = \Delta V * G$$

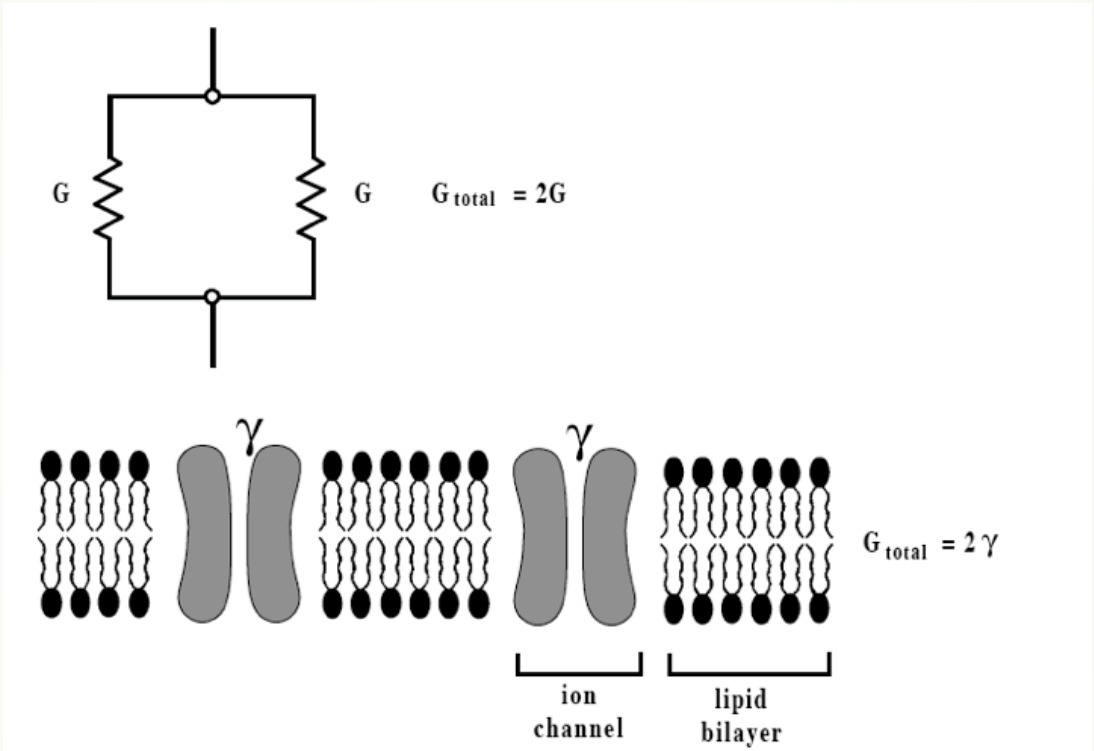


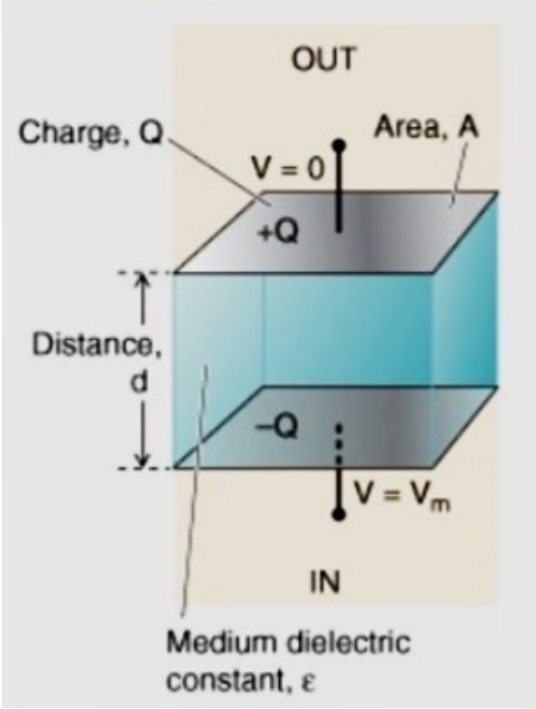
Figure 1-3. Summation of Conductance

Conductances in parallel summate together, whether they are resistors or channels.

Membrane capacitance determines the ability to separate charges of opposite sign

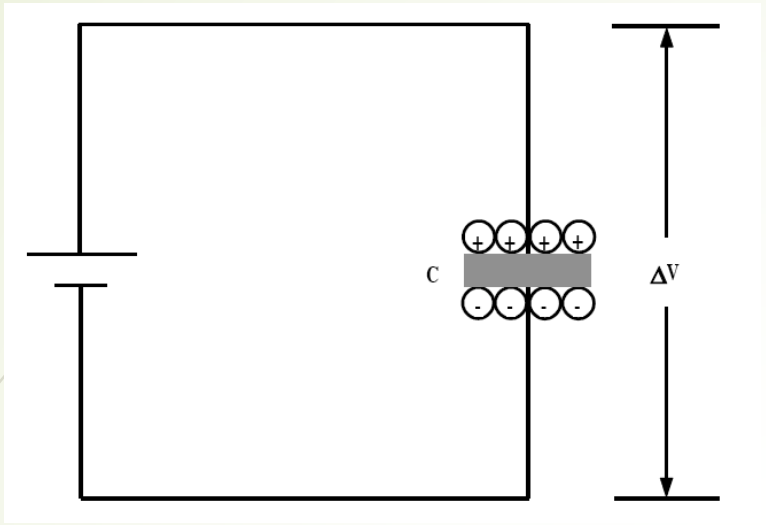
$$C_m = \frac{\varepsilon A}{d}$$

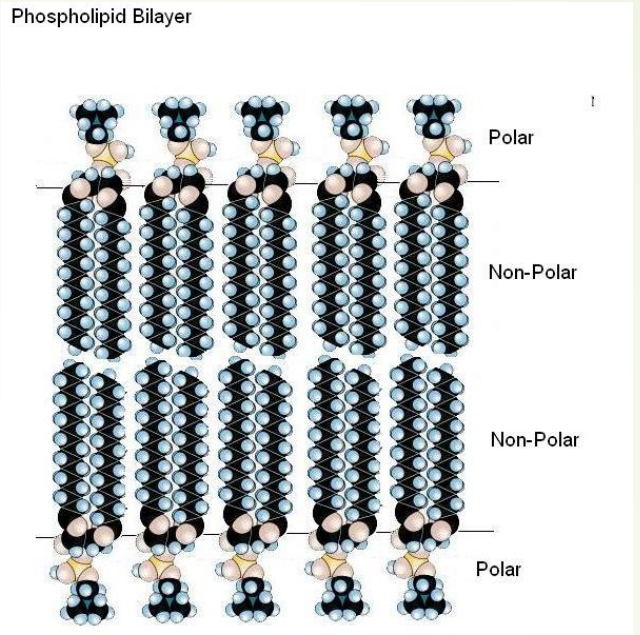
ε=dielectric constant



The charge stored in a capacitor is the product of capacitance and voltage

$$Q = C\Delta V$$





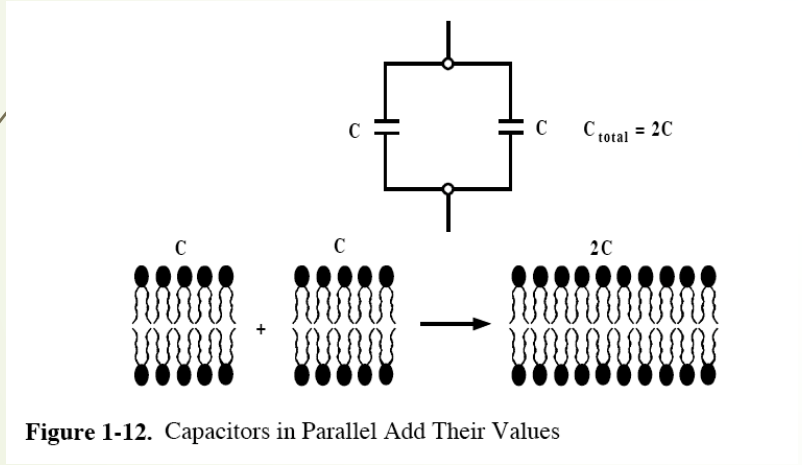


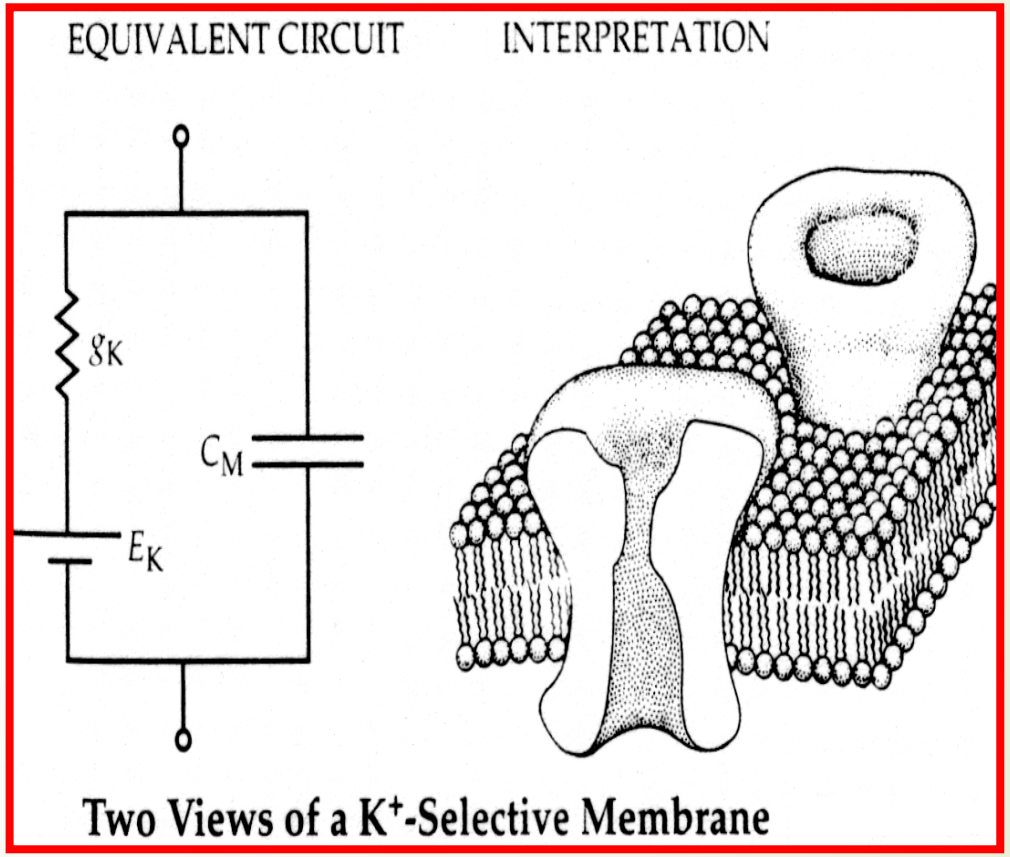
Figure 1-12. Capacitors in Parallel Add Their Values

When multiple capacitors are connected in parallel, this is electronically equivalent to a single large capacitor; that is, the total capacitance is the sum of their individual capacitance values (Figure 1-12). Thus, membrane capacitance increases with cell size. Membrane capacitance is usually expressed as value per unit area; nearly all lipid bilayer membranes of cells have a capacitance of 1 μF/cm^2 (0.01 pF/μm^2).

$$I_m = I_i + I_c$$

$$I_i = G(V_m - E_{ion})$$

$$I_c = \frac{\Delta Q}{t} = \frac{C_m \Delta V_m}{t}$$

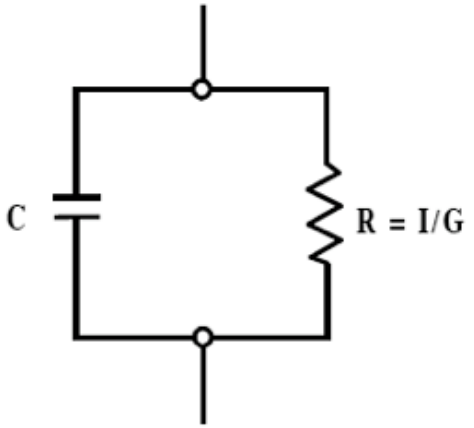


Two Views of a K⁺-Selective Membrane

$I_m = I_c + I_i$

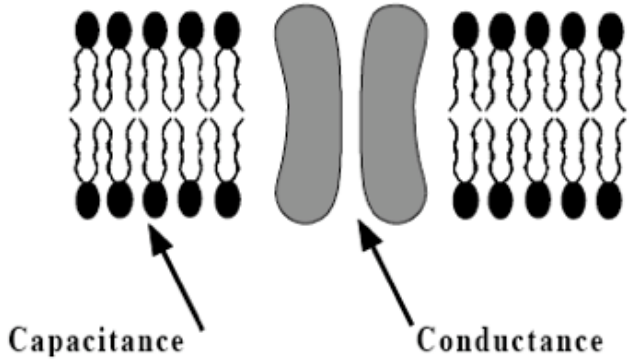
V_m=membrane potential (Volts)
V_{eq}=equilibrium potential (for an ion) (Volts)
C_m=membrane capacitance (Faraday)
G=conductance (Siemens)
R=resistance (Ohm)

$$I_c = \frac{\Delta Q}{t} = \frac{C_m \Delta V_m}{t}$$



$$I_i = G(V_m - V_{eq})$$

$$V_{eq} = \frac{RT}{zF} \log \frac{C_{out}}{C_{in}} \quad \text{NERNST}$$



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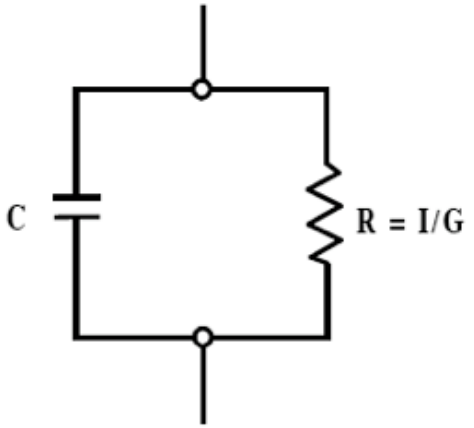

$$I_i = G(V_m - V_{eq})$$

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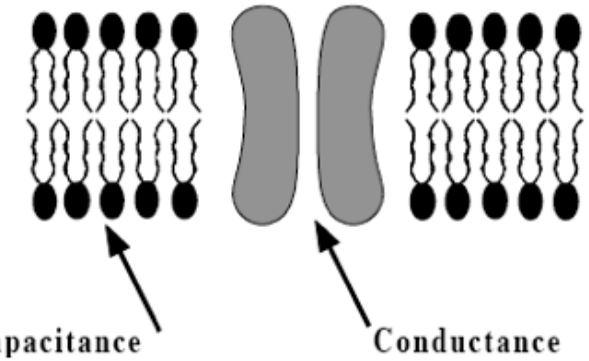
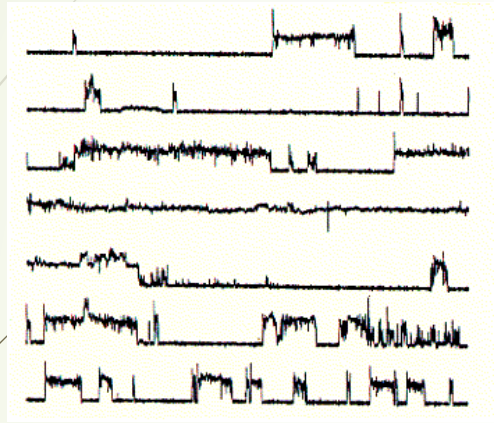
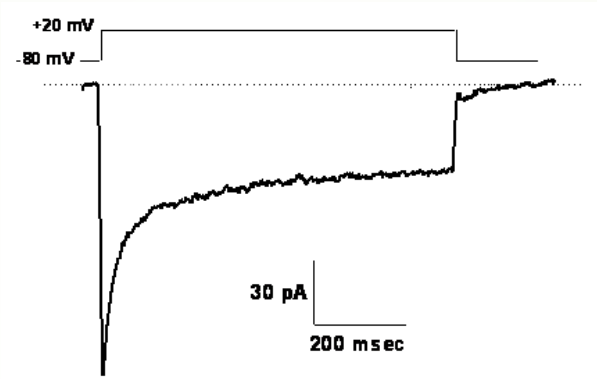
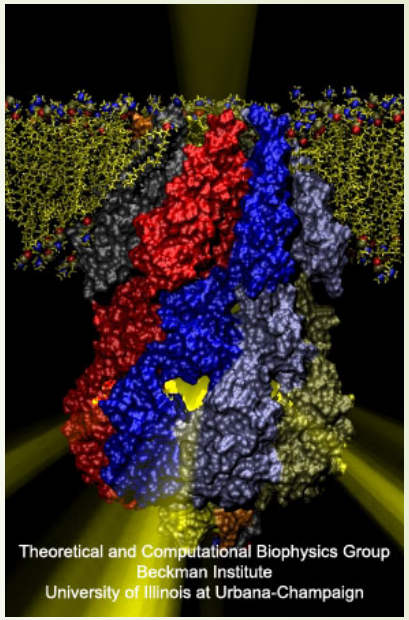


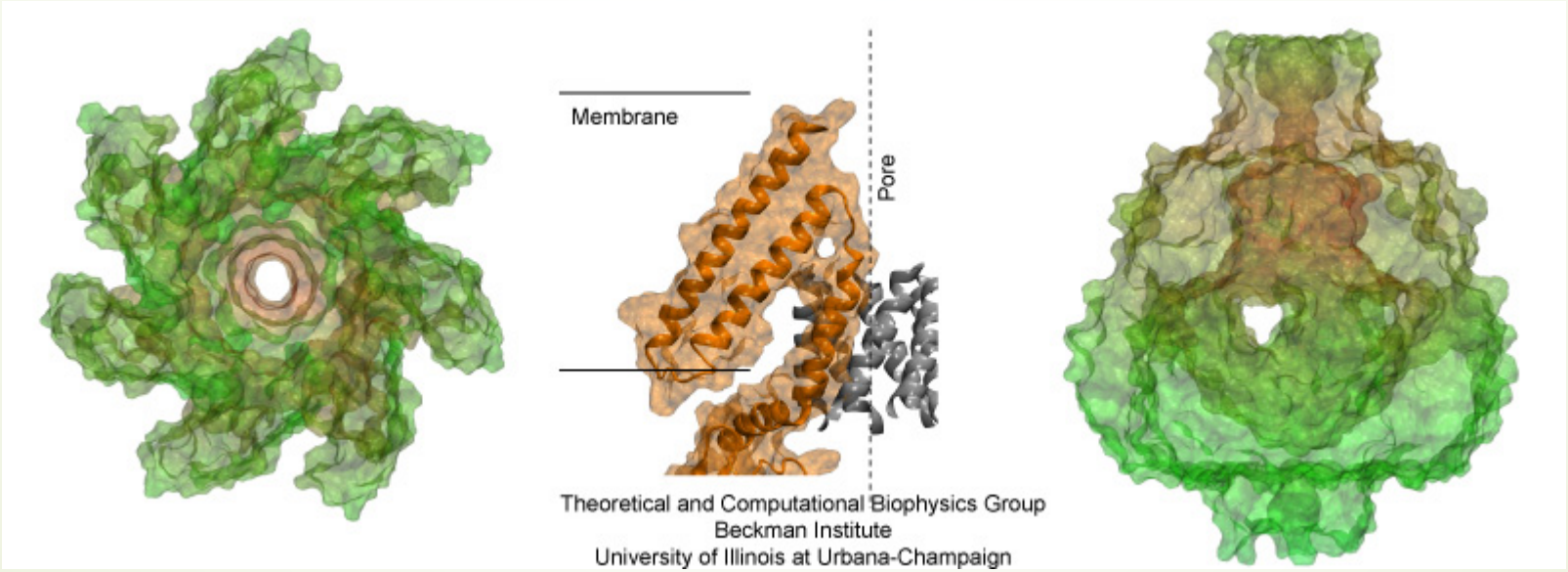
$$V_m = \frac{RT}{F} \ln \frac{P_K[K^+]_e + P_{Na}[Na^+]_e + P_{Cl}[Cl^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_e} \quad \text{GOLDMAN-HODGKIN-KATZ}$$

The variation of the membrane potential generates electrical signals due to the membrane potential variation in the time unit







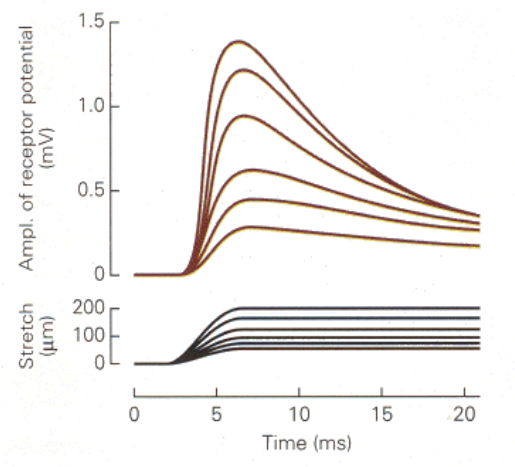


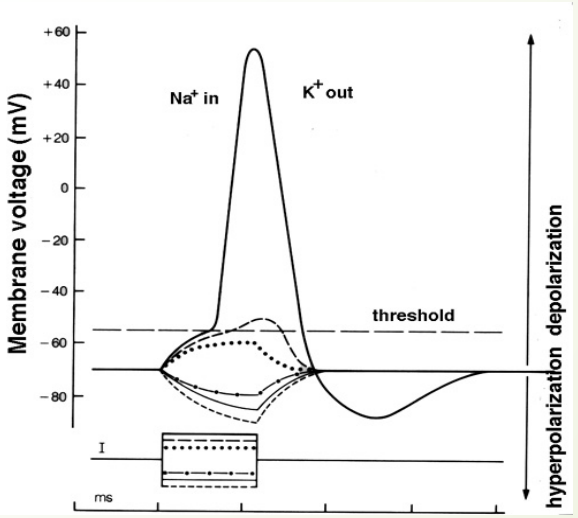
Electrotonic potential (EPSP; IPSP)

- graded
- local (propagation with exponential decay)
- integration
- depolarization/hyperpolarization

Action potential

- all or none
- long distance propagation
- always a depolarization





Functions

Electro tonic potential

- Sensorial systems: receptor potential
- Chemical synapses: postsynaptic potential
- Amplitude codification

Action Potential

- Muscle contraction
- Long distance communication
- Secretion (Exocytosis)
- Frequency codification

EPSP Vs ACTION POTENTIAL:

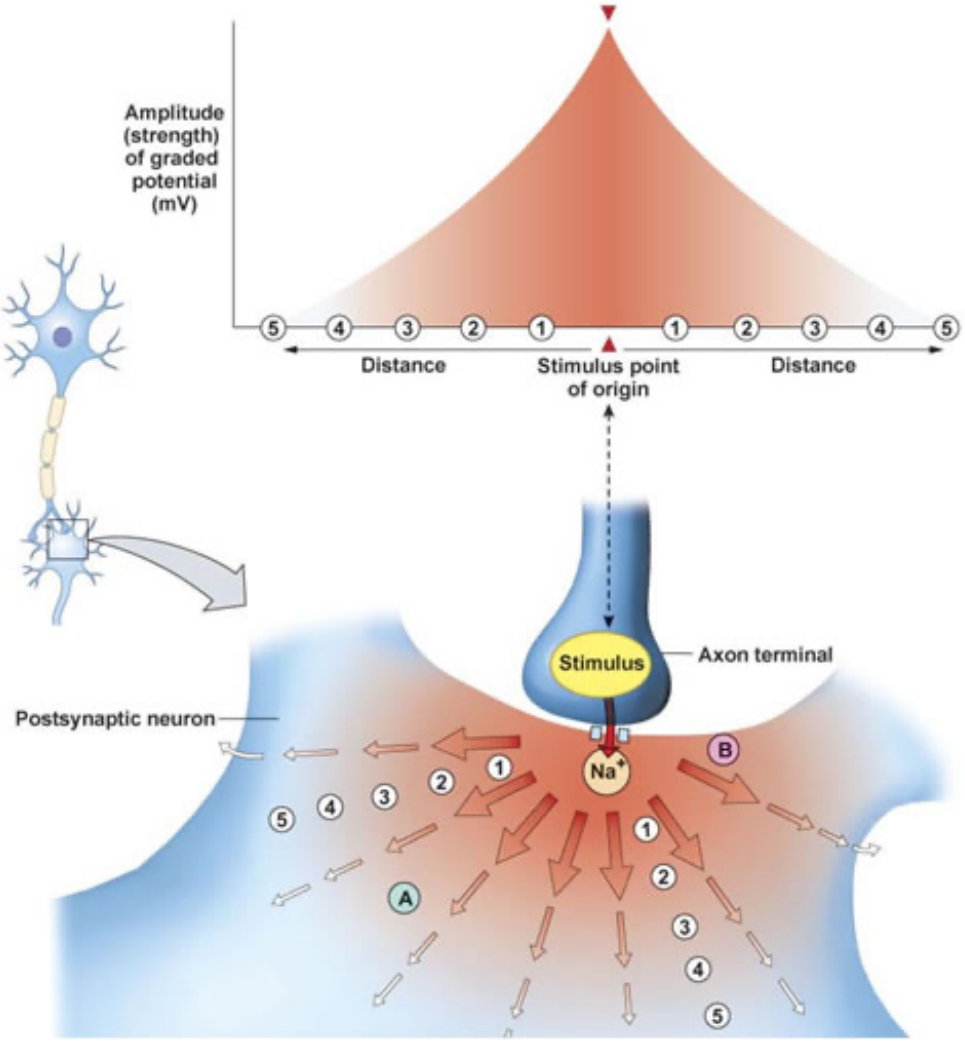
Property	EPSP or IPSP or Graded potential	Action Potential
Magnitude	Low	High
Propagation & Duration	Nil; it remains localized (up to 20 msec)	Self propagating (up to 2 msec)
Refractory period	absent	present
All or none law	Not obeyed. It is graded.	obeyed
Summation	Present	absent
Decrement (decline of size with distance)	present	Absent. Size is constant
Increased permeability to ions	To Na+ & K+ at one time but Na+ influx >	Na+ Influx , then K+ efflux

GRADED POTETIALS

- In neurons are generally located at the level of the dendrites or cell body.
- Positive potentials (depolarizing, EPSP) or negative potentials (iperpolarizing, IPSP)
- GRADED because their amplitute is proportional to the generating event: a strong stimulus will generate a graded potential of big amplitude, a light stimulus will generate a smaller amplitute potential.
- In the Nervous system graded potentials are generated by the release of neurotransmitters from chemical synapses, by electrical signals from electrical synapses or by the closure of ion channels

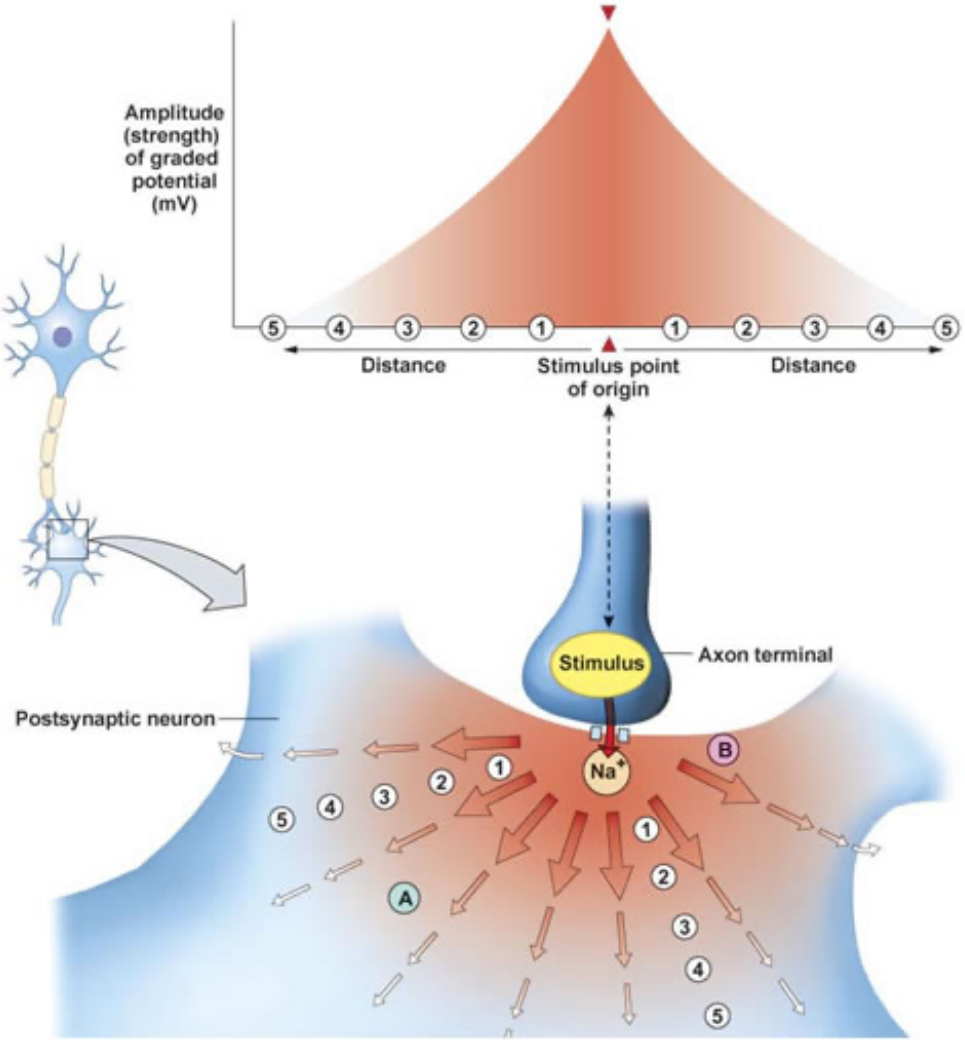
GRADED POTETIALS

- Due to the synaptic release, the neurotransmitter activates Na+ permeable channels
- Na+ wil follow its electrochemical gradient entering the cell and depolarizing the membrane.
- The depolarization will diffuse within the cytosol creating a local current



GRADED POTETIALS

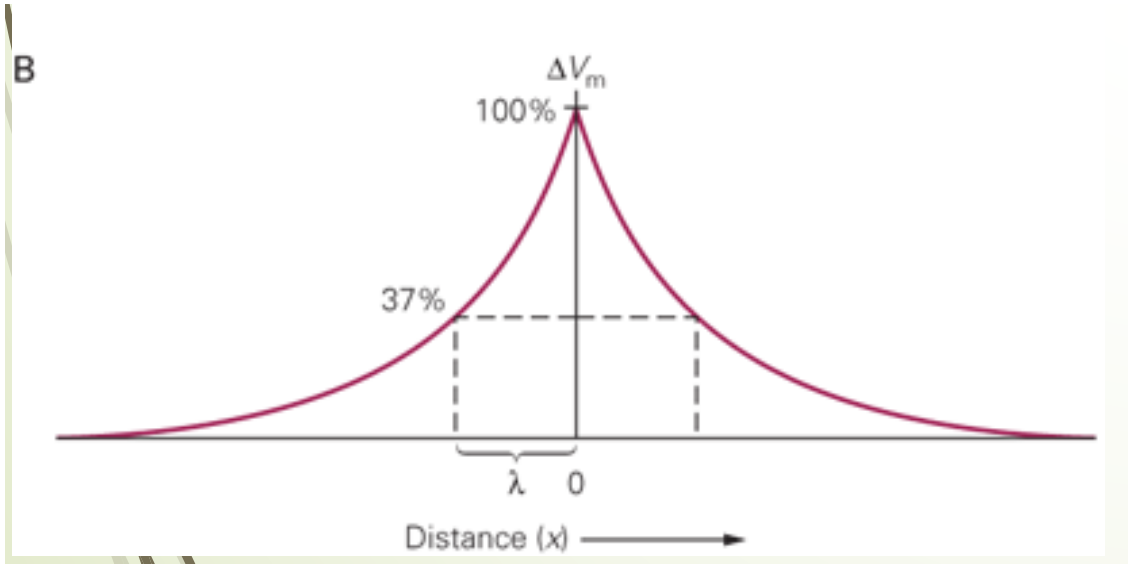
- The initial intensity of the depolarization depends on the amount of + charge flowing in the cell: the more Na+ permable channels will open the more intense will be the current generated and as a consequence the bigger will be the initial amplitude of the graded potential
- The bigger is the initial amplitude the bigger the distance the graded potential will diffuse



GRADED POTETIALS

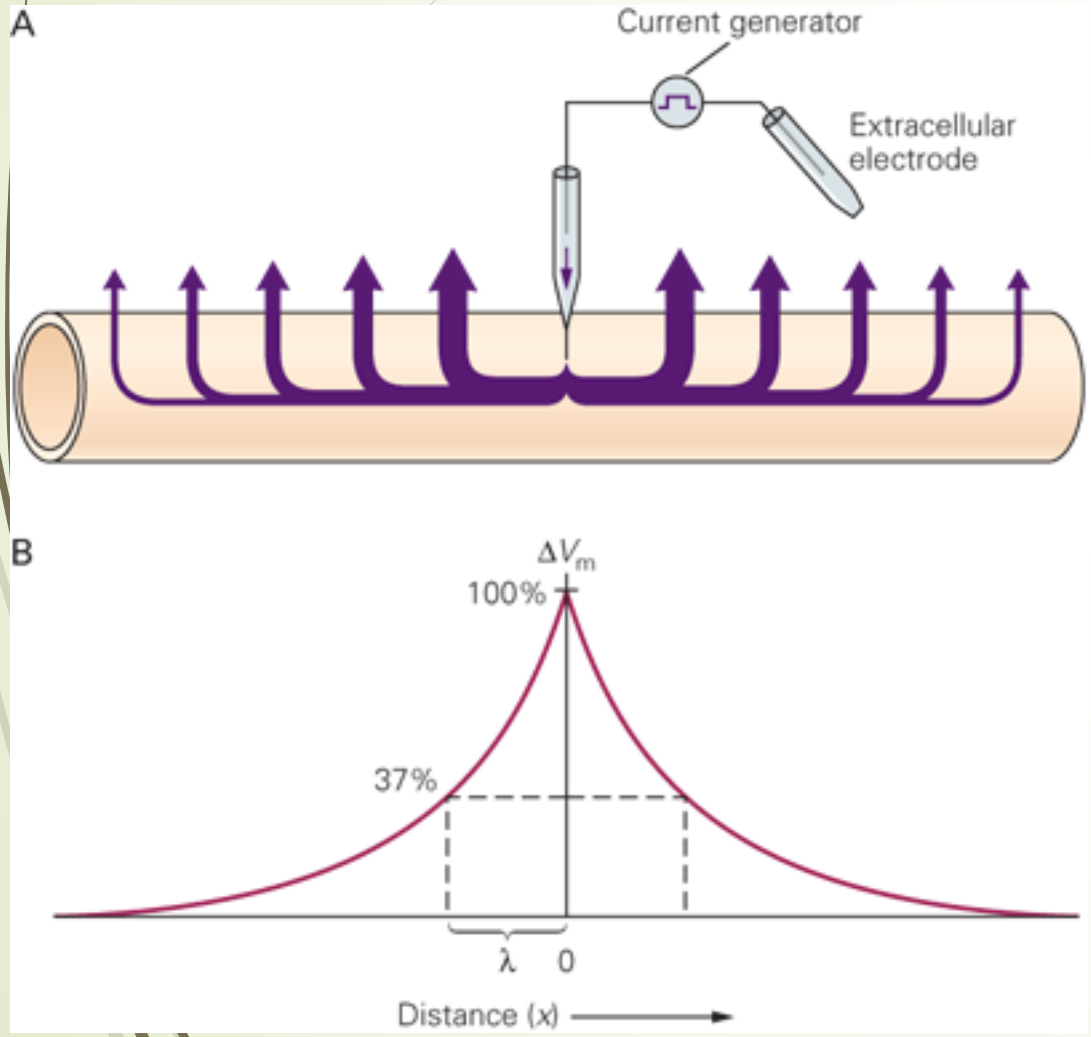
- The graded potential follows an exponential decay as a function of the distance

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



Propagation of signal conduction

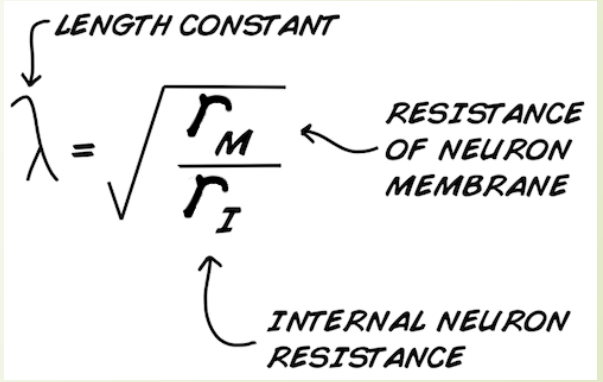
How geometry influences the distribution of current



The variation of the Vm with distance depends on the relative value of the **membrane resistance** in a unit length of dendrite, $\mathbf{r_m}$ (units Ω * cm) and internal neuron resistance per unit length of the dendrite, $\mathbf{r_i}$ (units Ω/cm).

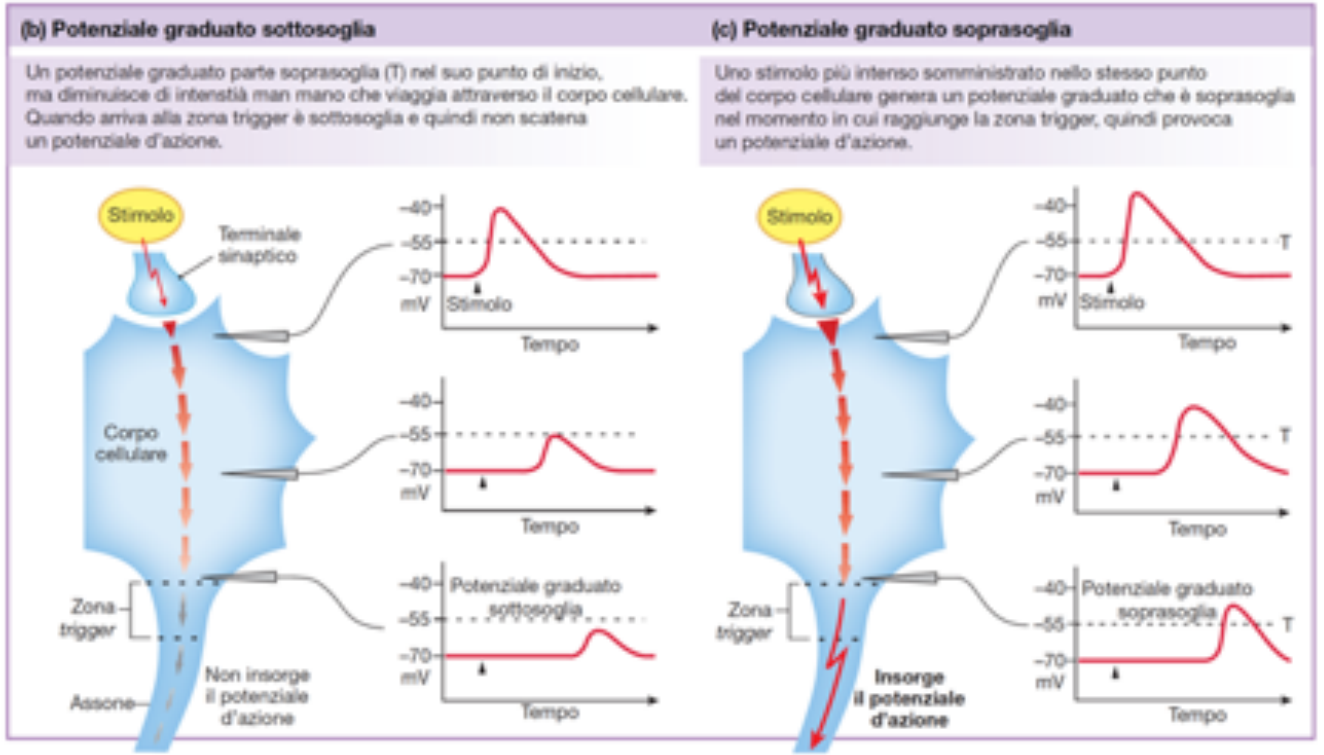
The change in Vm becomes smaller with distance along the dendrite away from the electrode. The decay with distanceis exponential:

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



GRADED POTENTIALS

- The combination of r_m and r_i will give rise to the spatial decay of the graded potential
- If the graded potential is intense enough to reach the **«trigger zone»** over the threshold, then an Action Potential will be generated.



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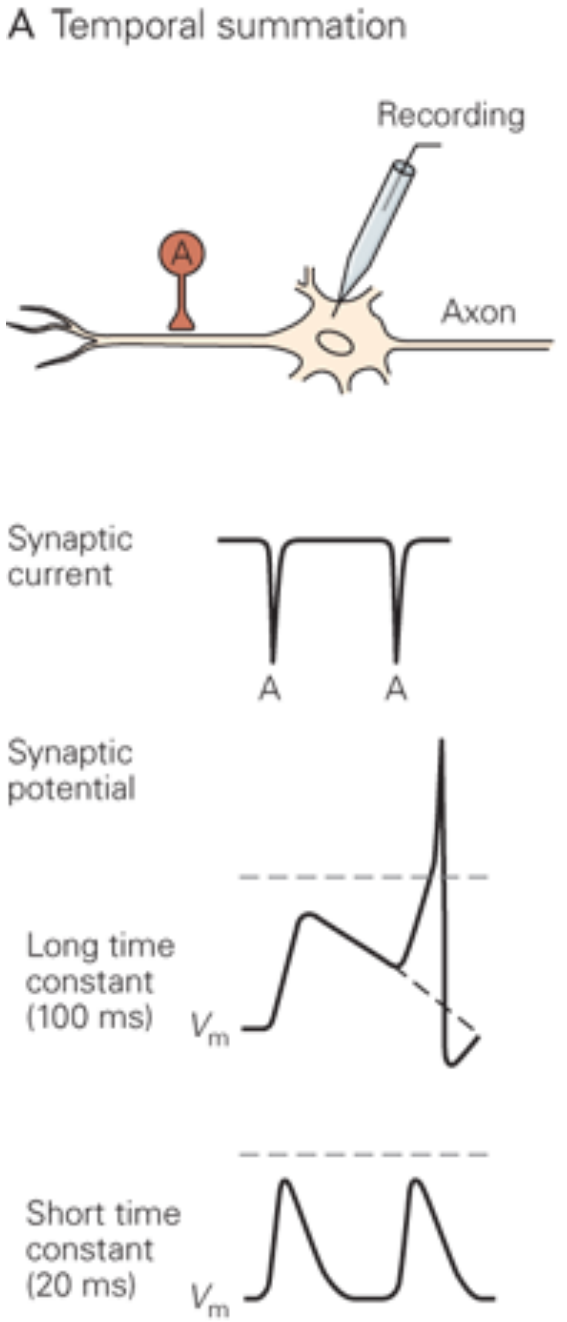
Neuronal integration involves the summation of synaptic potentials that spread to the trigger one, it is clinically affected by two passive membrane properties of the neuron:

- **Membrane time constant** helps determine the time course of the synaptic potential controlling **temporal summation,** the process by which consecutive synaptic potential at the same site are added together in the postsynaptic cell.

Neurons with a large membrane constant have a greater capability for temporal summation than do neurons with a shorter time constant

The longer the time constant of the membrane, the **greater the likelihood** that **two consecutive inputs from an excitatory presynaptic neuron will summate** to bring the cell membrane to its threshold for action potential

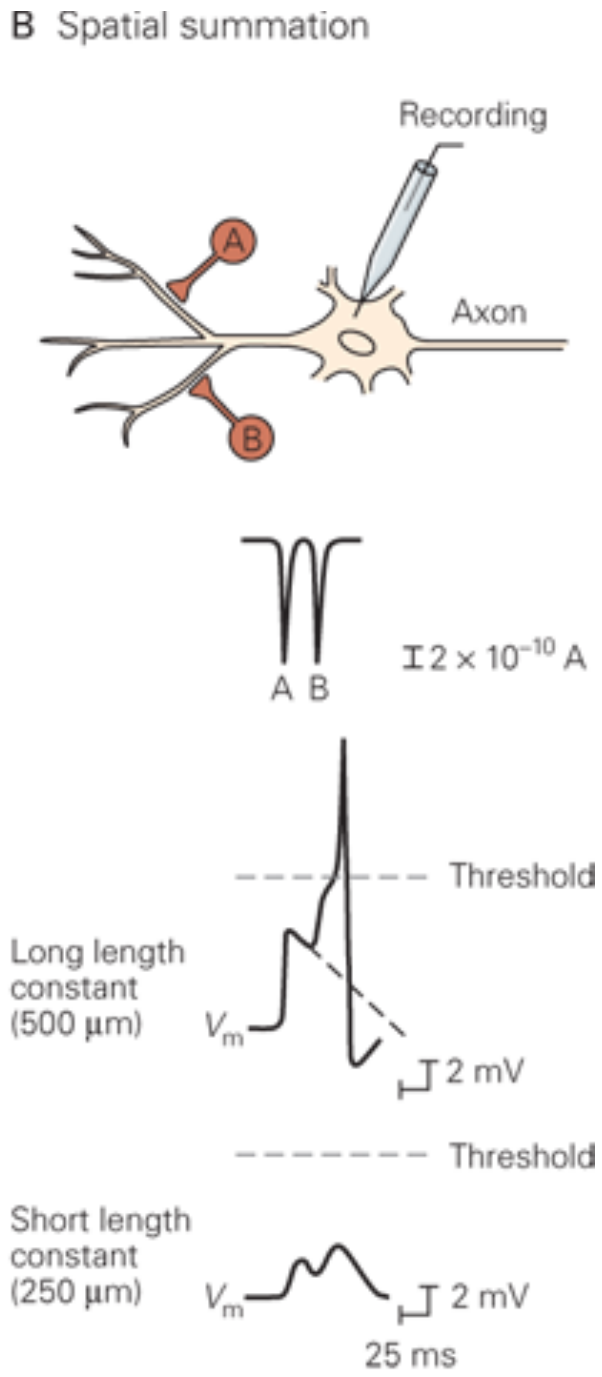


- **Length constant** of the cell determines the degree to which a local depolarization decreases as it spreads passively from a synapse along the length of the dendrite.

In cells with longer length constant, signals spread to trigger zone with minimal decrement; in cells with a short length constant, the signal decay rapidly with distance

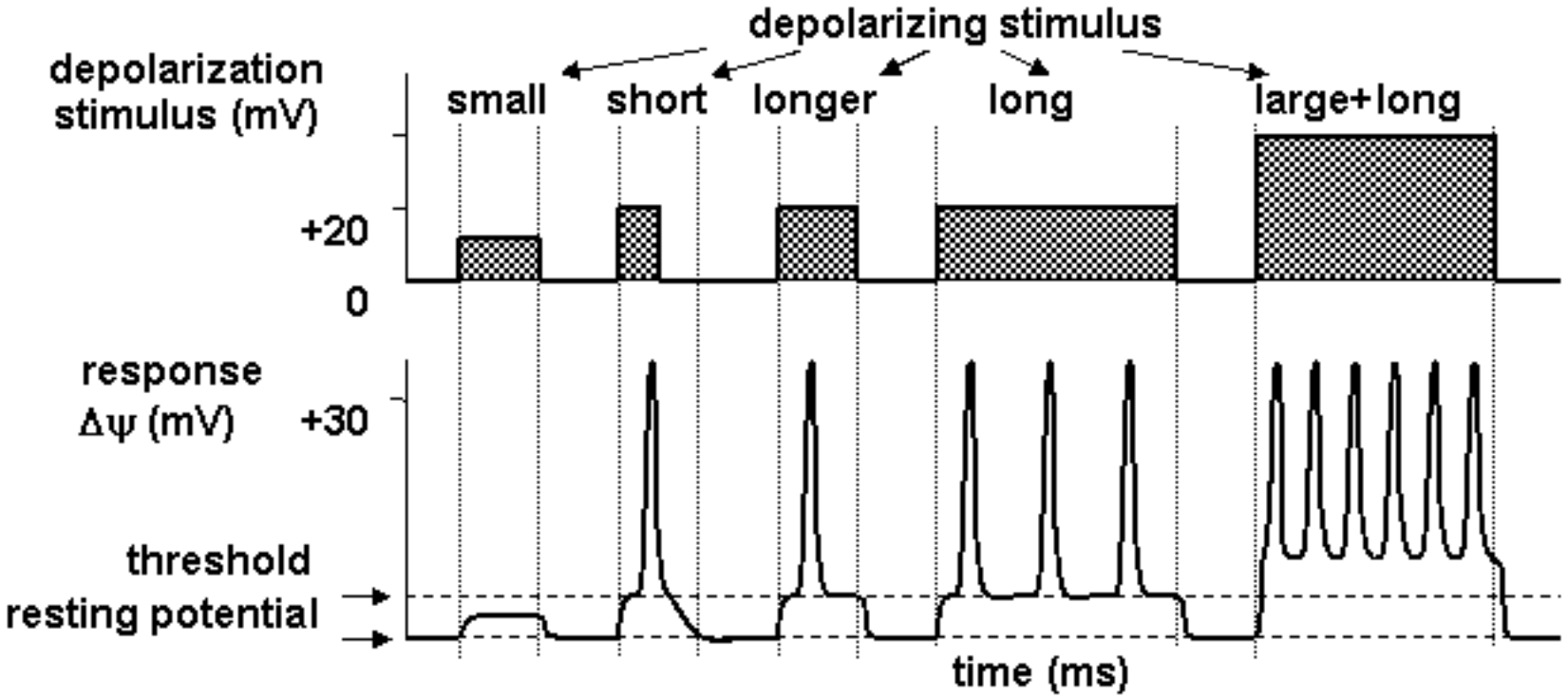
Inputs from many presynaptic neurons acting at different sites of the postsynaptic neuron must be added together: **spatial summation.**

Neurons with larger length constant are more likely to be brought to threshold by inputs arising from different sites than are neurons with a short length constant



Amplitude-frequency coding

- Amplitude of the stimulus due to the signal integration influences the action potential frequency rate which represent the neuron coding signal



Cellular Excitability

Require: high expression of Voltage-gated channels

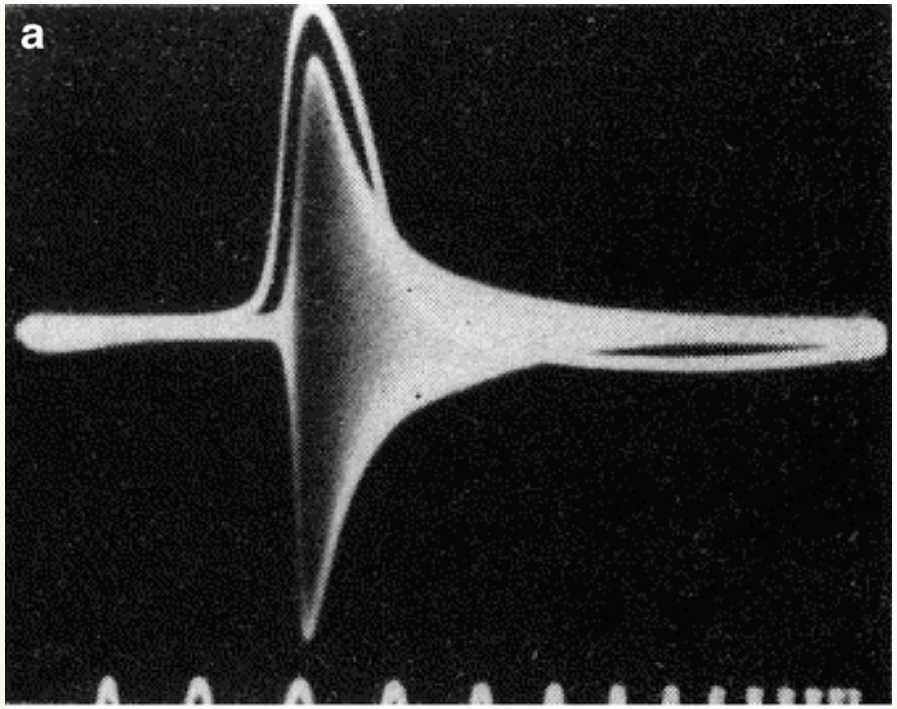
Ability to generate Action Potential
If stimulated

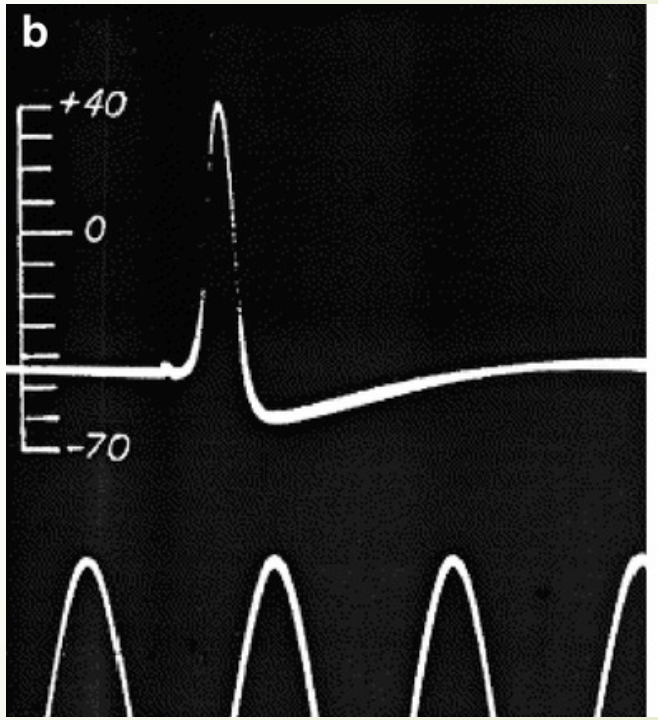
Excitable cells:
Neurons
Muscle (striated and smooth)
Secreting cells

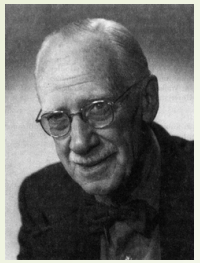
Some cells are auto excitable: they don't need external stimuli to promote Action Potential **(pacemaker cells in the heart, neurons)**

Action Potential phases

Hodgkin and Huxley (1939):
Classical studies on giant Squid Axon intracellular measurements



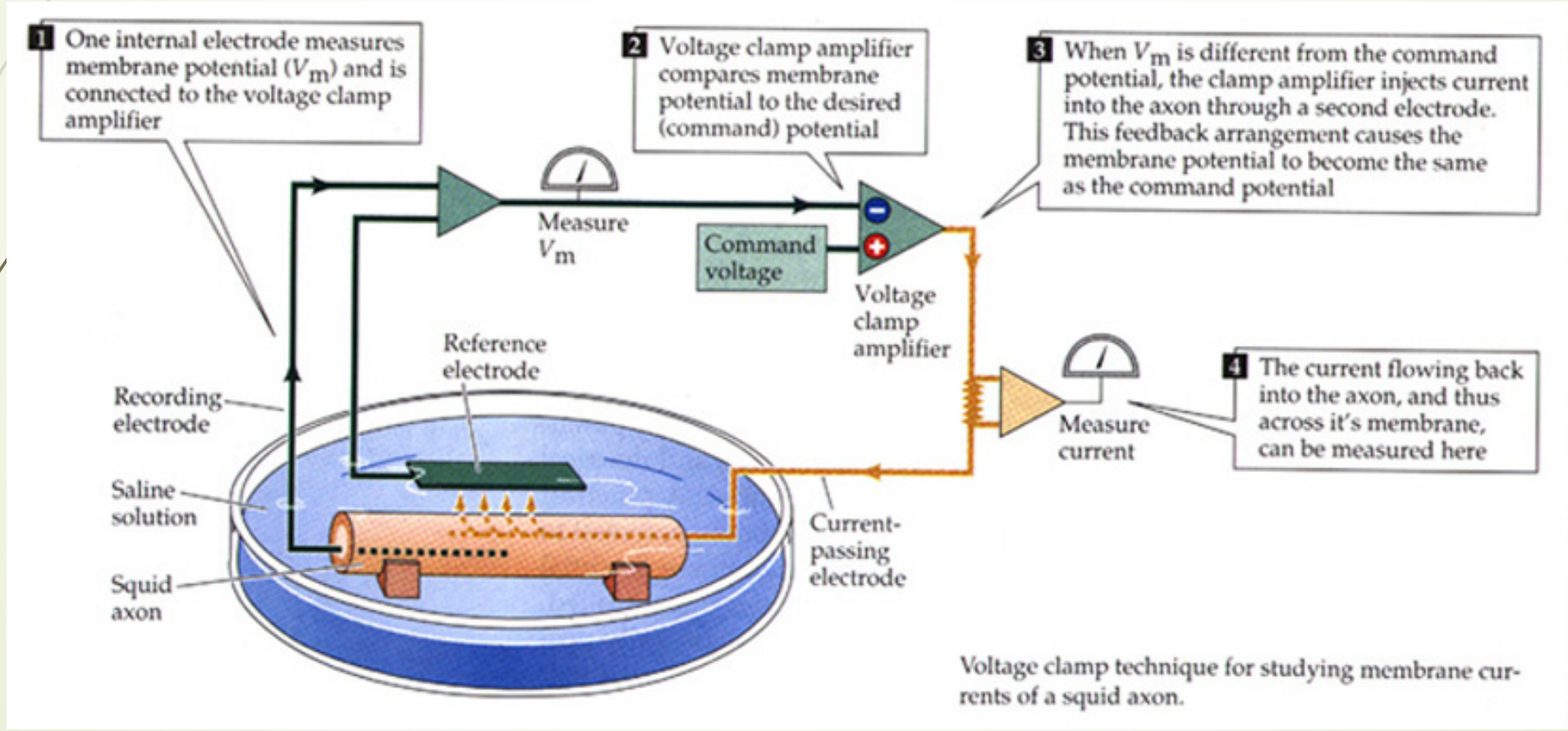




Cole ('47)

VOLTAGE CLAMP technique

Quantitative analysis of ionic currents 'blocking' membrane voltage at a given value.

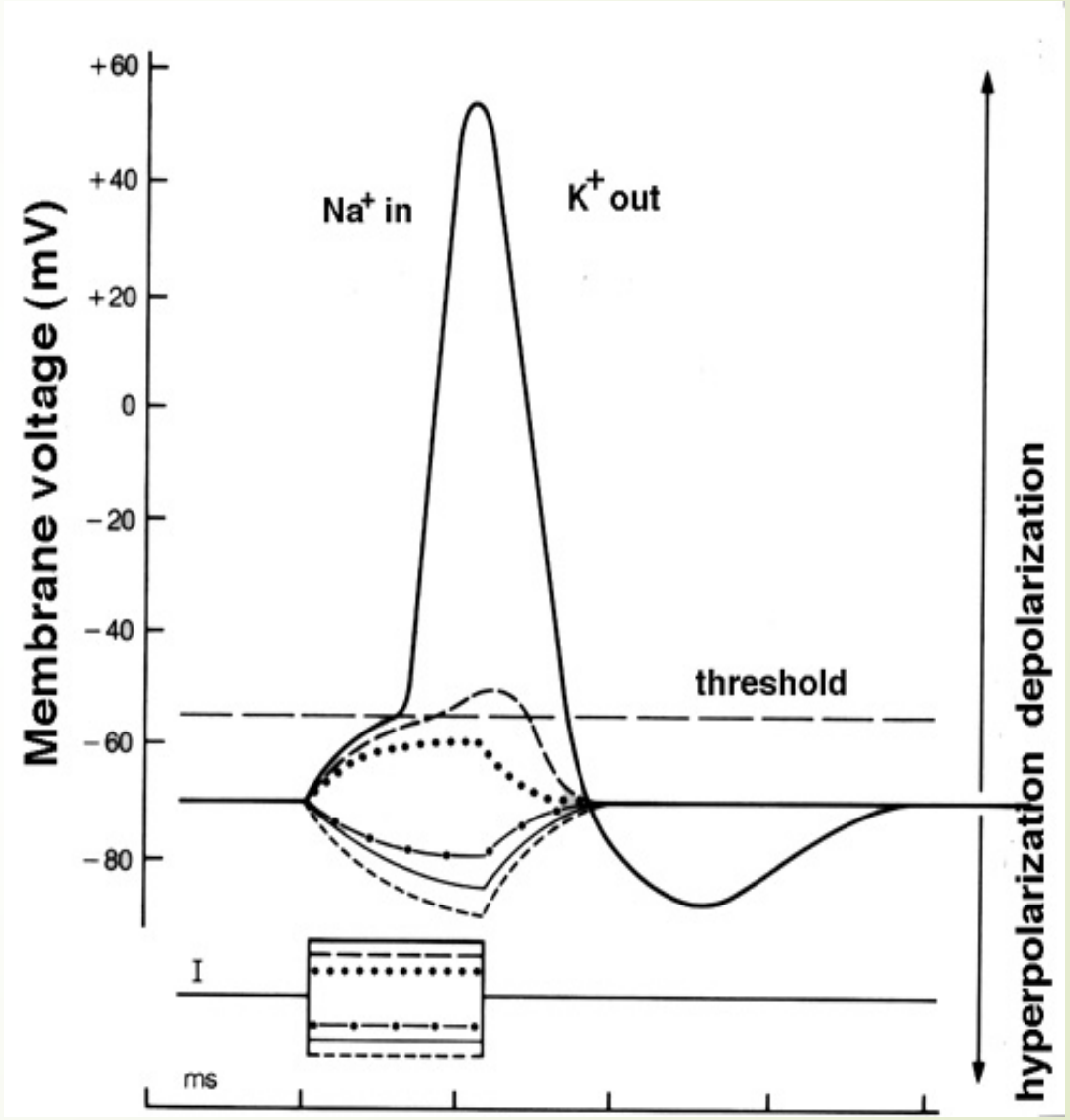


Voltage clamp technique for studying membrane currents of a squid axon.

Action Potential phases

Hyperpolarizing stimuli:
The responses are similar to a RC circuit.

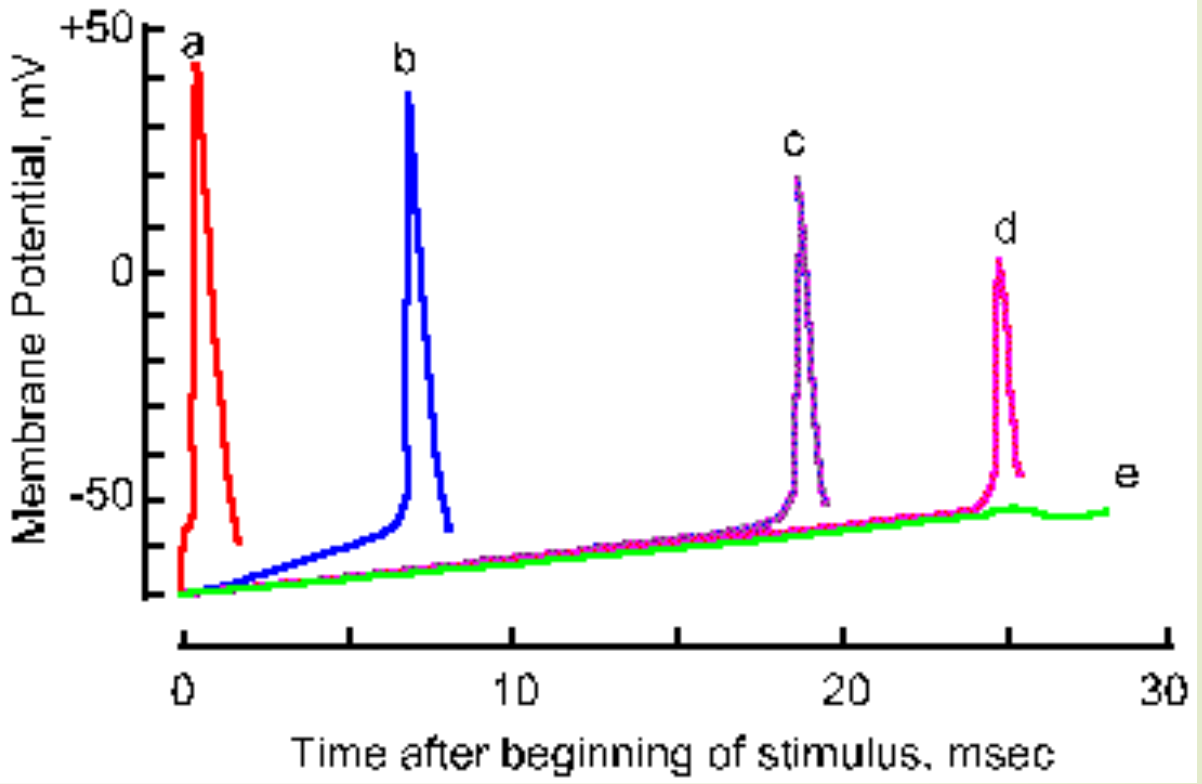
Depolarization with sufficient intensity:
Action Potential = fast membrane changes to 0 and over (OVERSHOOTING) reaching about +40mV.
Fast repolarization phase under the resting initial potential (UNDERSHOOT)
TOT DURATION in giant squid axon = few milliseconds



Action Potential phases

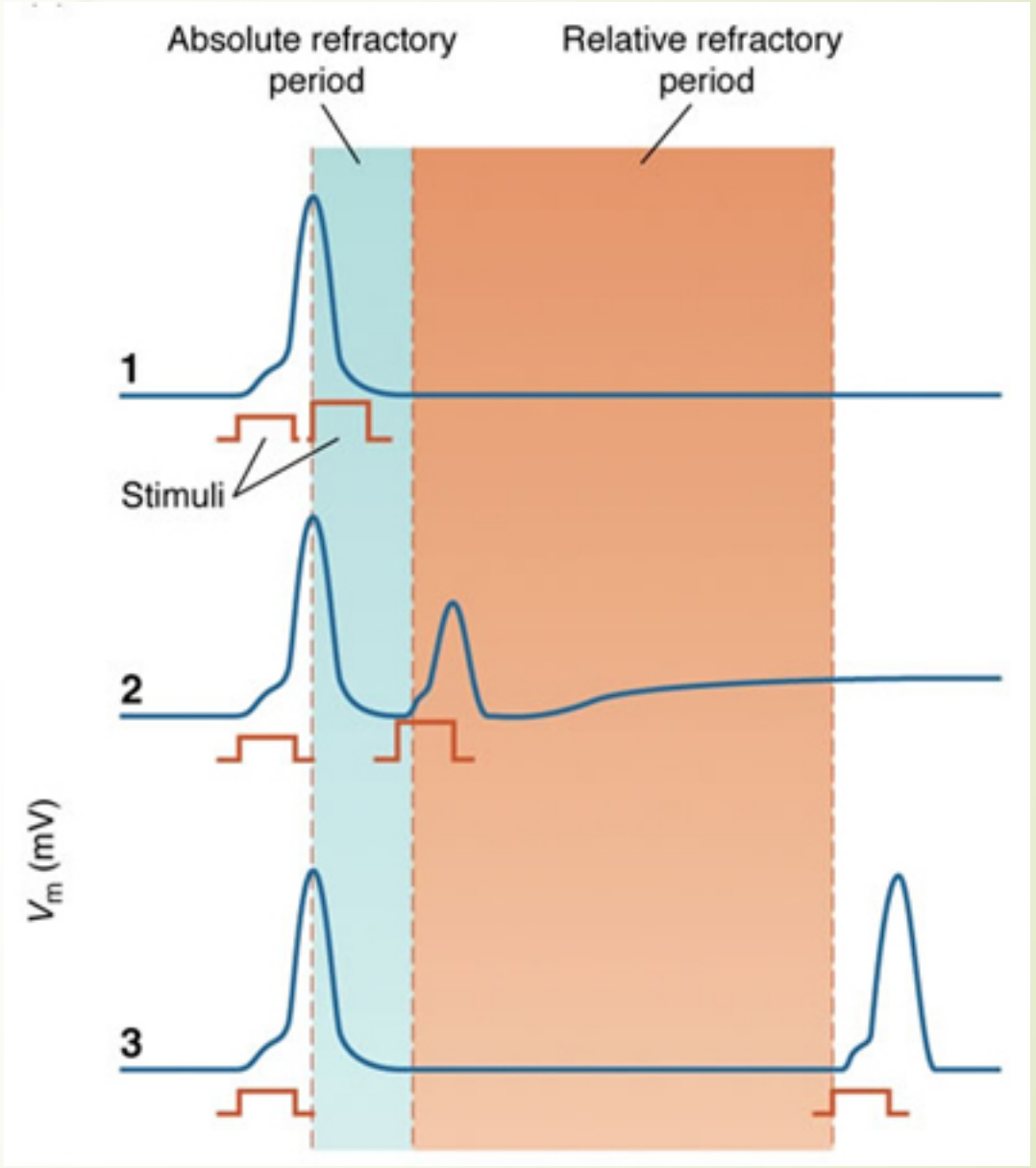
Action Potential is evoked when the Vm reaches a THRESHOLD value. This value is not a constant but can be changed by changing the stimulating conditions

If the stimuli changes linearly rather than in steps, we can observe an increase in the threshold: **ACCOMODATION** due to the inactivation of Na^+_v



Action Potential phases

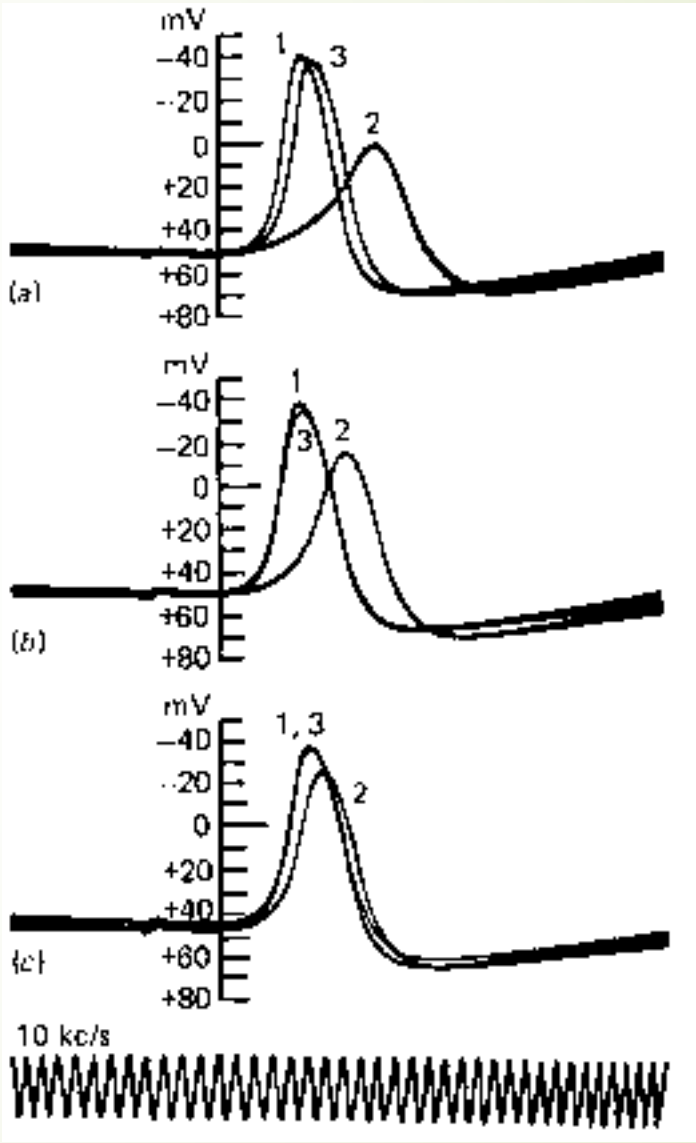
Na^+_v inactivation is also responsible for the **REFRACTORY PERIOD.** Beside the fast processes regulating the action potential in ms, there is a slower process without visible effect on the potential but necessary to recover the axon to the initial excitability conditions



The Sodium hypothesis

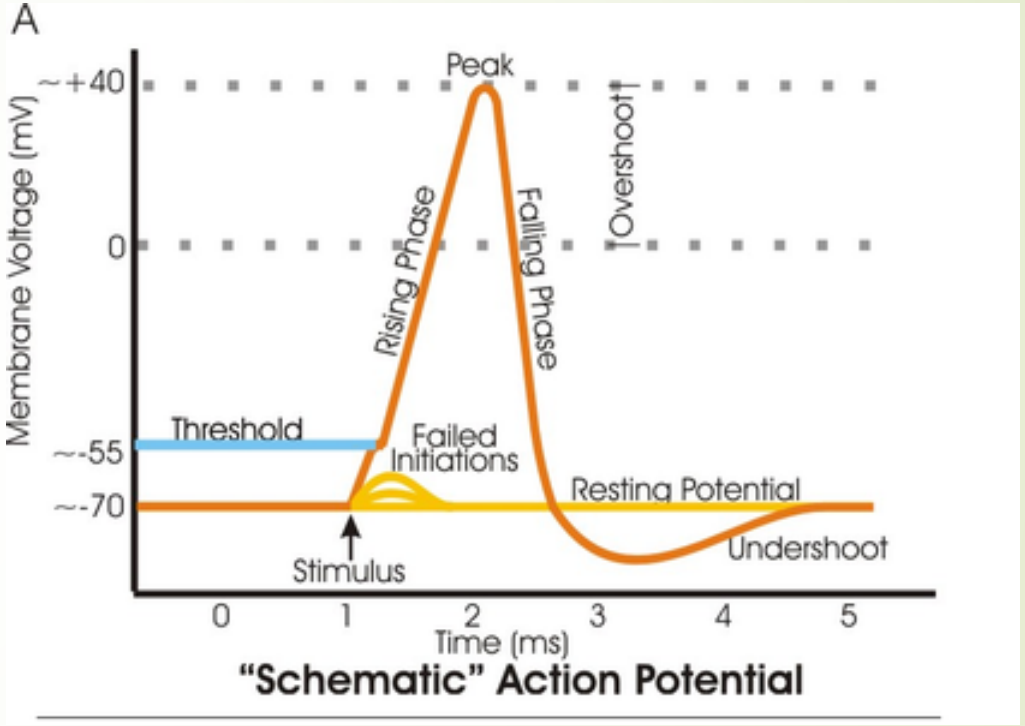
Hodgkin and Katz described in 1949 the Dependency of the **OVERSHOOT** from Na+

The effect of **reducing the external sodium** concentration on the action potential in a squid giant axon. In each set of records, record 1 shows the response with the axon in sea water, record 2 in the experimental solution, and record 3 in sea water again. The solutions were prepared by mixing sea water and an isotonic dextrose solution, the proportions of sea water being a, 33%; b, 50%; c, 71%. From Hodgkin and Katz (1949).



The Sodium hypothesis

From these experiments emerged the idea that the membrane becomes more permeable to Na+ as compared to K^+ and therefore V_m tends to V_{Na}^+.
The peak of the action potential in fact always close to V_{Na}^+ (=+40)



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Thank you