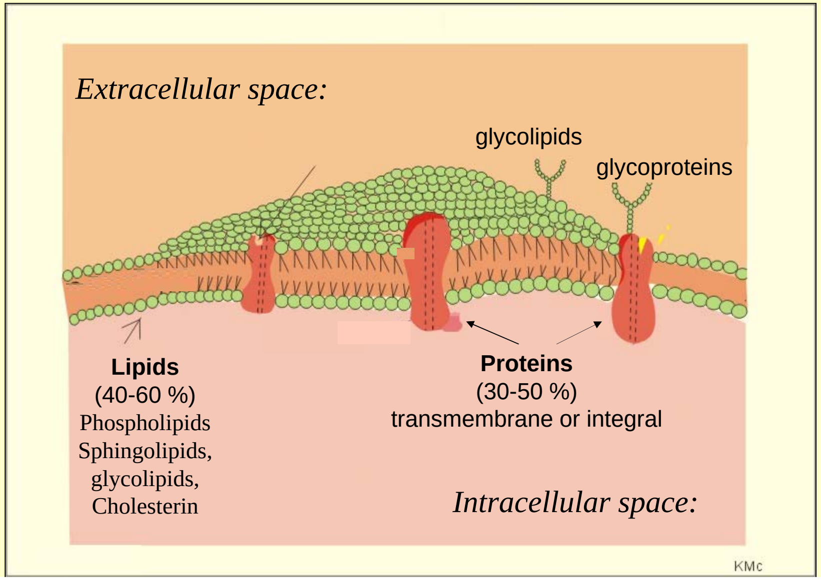
Transport across biological membranes

Transport in Resting Cell

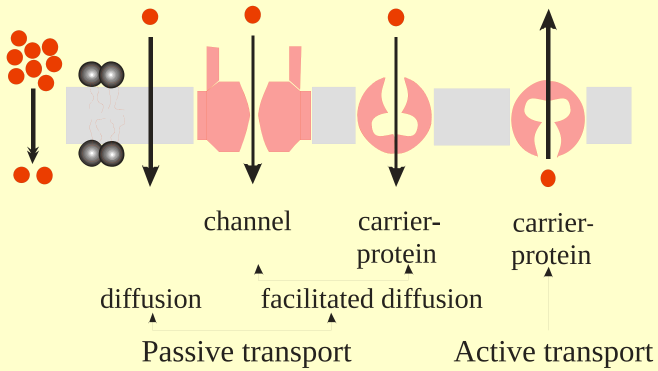
Membrane structure



Transport types across the memebranes

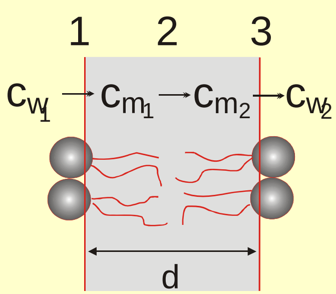
Classification based on: energy consumption

molecular mechanism



Diffusion of neutral particles

Diffusion across the lipid bilayer



Assume that concentration changes linearly

Fick I.

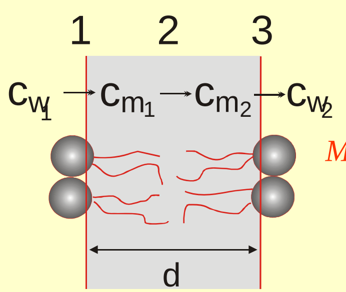
$$J_m = -D\frac{\Delta c}{\Delta x}$$

$$D_m << D$$

$$J_m = -D_m\frac{c_{m_2} - c_{m_1}}{d}$$

Diffusion of neutral particles

Diffusion across the lipid bilayer



$$J_m = -D_m \frac{c_{m_2} - c_{m_1}}{d}$$

$$J_m = -p_m (c_{m_2} - c_{m_1})$$

Membrane permeability constant [ms^{-1}]

Cannot be measured

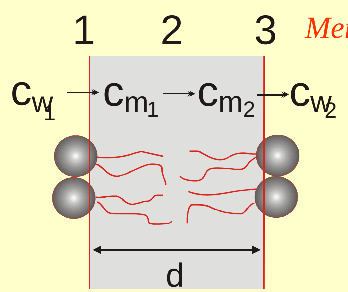
$$\frac{C_{m_1}}{C_{v_1}} = \frac{C_{m_2}}{C_{v_2}} = K$$

$$C_{m_1} = KC_{v_1}$$

Diffusion of neutral particles

Diffusion across the lipid bilayer

$$J_m = -p_m (c_{m_2} - c_{m_1})$$



Membrane permeability constant [ms-1]

Cannot be measured

$$\frac{C_{m_1}}{C_{v_1}} = \frac{C_{m_2}}{C_{v_2}} = K$$

$$C_{m1} = KC_{v1}$$

$$J_m = -p_m K (c_{v_2} - c_{v_1})$$

$$J_m = -p (c_{v_2} - c_{v_1})$$

$$J_m = -\mathbf{p}(C_{v2} - C_{v1})$$

Permeability constant [ms^{-1}]

It is influenced by:

- diffusion coefficient within the membrane
- thickness of the membrane
- partition coefficient

Permeability vs hydrophobicity

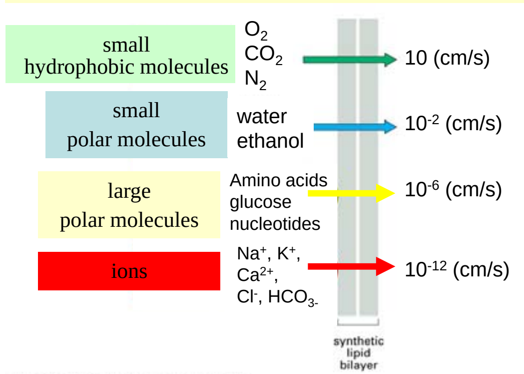
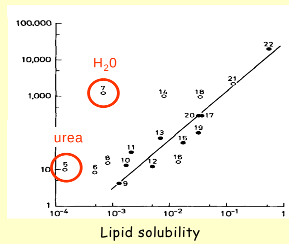


Figure 12-2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Lipid solubility v permeability



Diffusion of ions

$$\text{Fick I.} \quad J_m = -D\frac{\Delta c}{\Delta x}$$

chemical potential
and
electric potential
together

$$J_k = L_k X_k = -L_k \frac{\Delta \mu_{ek}}{\Delta x}$$

flux of *k*-th ion

Diffusion of ions

$$J_k = L_k X_k = -L_k \frac{\Delta \mu_{ek}}{\Delta x}$$

$$\frac{\Delta \mu_{ek}}{\Delta x} = \frac{\Delta \mu_k}{\Delta x} + Z_k F \frac{\Delta \varphi}{\Delta x} \qquad \text{és} \qquad L_k = c_k \frac{D_k}{RT}$$

$$J_k = -D_k \left(\frac{\Delta c_k}{\Delta x} + c_k \frac{z_k F}{RT} \frac{\Delta \varphi}{\Delta x} \right)$$

$$D = ukT$$

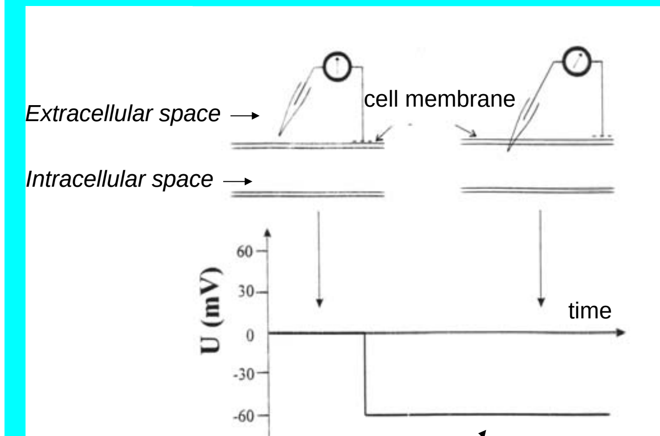
$$J_k = -u_k kT \left(\frac{\Delta c_k}{\Delta x} + c_k \frac{z_k F}{RT} \frac{\Delta \varphi}{\Delta x} \right)$$

flux of *k*-th ion

Basic principles of electrophysiology

Interpretation by transport phenomena

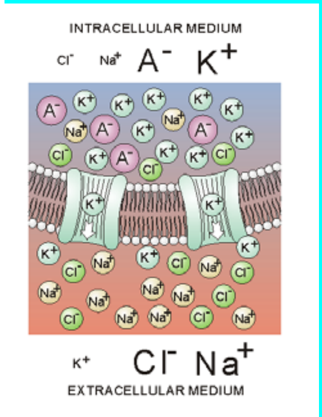
Observation 1: There is an electric potential difference between extra- and intracellular space



The intracellular side is negative with respect to the extracellular side

resting potential ~ 60 – 90 mV

Observation 2: Inhomogeneous ion distribution



Cell type	$C_{Intracellular}$ (mmol/l)			$C_{Extracellular}$ (mmol/l)		
	$[Na^+]_i$	$[K^+]_i$	$[Cl^-]_i$	$[Na^+]_e$	$[K^+]_e$	$[Cl^-]_e$
Squid axon	72	**345**	61	**455**	10	540
Frog muscle	20	**139**	3,8	**120**	2,5	120
Rat muscle	12	**180**	3,8	**150**	4,5	110

Interpretation of the membrane potential

Model 1

Constant ion distribution in resting state

↓

No transport (?)

↓

Assume that (1) the system is in ***equilibrium***

that is

no electrochemical potential difference

$$\mu_{e,i}^{II} - \mu_{e,i}^{I} = 0$$

$$\mu_{e,i}^{II} - \mu_{e,i}^{I} = 0$$

↓

$$\mu_0 + RT\ln c_i^I + zF\varphi_i^I = \mu_0 + RT\ln c_i^{II} + zF\varphi_i^{II}$$

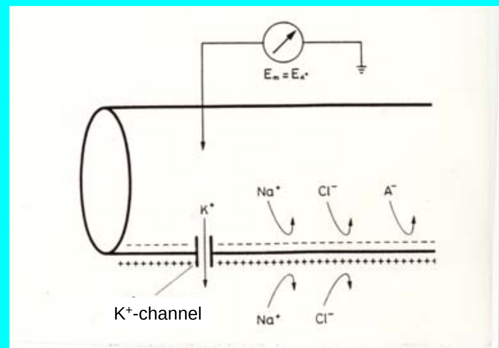
↓

Equilibrium potential → $\varphi_i^I - \varphi_i^{II} = \frac{RT}{zF} \ln \frac{c_i^I}{c_i^{II}}$

Nernst-equation

Assume (2) unlimited ***K^+*** permeability

(3) zero ***Na^+*** permeability



Donnan model – Equilibrium model

- No electrochemical potential difference between extra- and intracellular medium
- The membrane is permeable only for K^+ (and Cl^-)
- The cell with its extracellular region is thermodynamically closed system

↓

equilibrium potential ≡ resting potential

$$\varphi_e - \varphi_i = \frac{RT}{F} \ln \frac{[K^+]_i}{[K^+]_e}$$

$$\varphi_e - \varphi_i = \frac{RT}{F} \ln \frac{[K^+]_i}{[K^+]_e}$$

Data from the equilibrium approach do not agree with the experiments

Tissue	Resting potential (mV)	
	calculated	measured
Squid axon	*91*	62
Frog muscle	*103*	92
Rat muscle	*92,9*	92

Calculations based on other ions

potential (mV)	**Squid axon**	**Rat muscle**
$U_{measured}$	**-62**	**-92**
$\mathbf{U_{0K+}}$	-91	-103
$\mathbf{U_{0Na+}}$	+47	+46
$\mathbf{U_{0Cl-}}$	-56	-88

There is no good agreement

Interpretation of the membrane potential

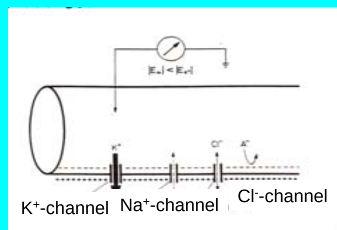
Model 2

1. Assume that the system is *not in **equilibrium***

that is

transport is forced across the membrane

2. Take into consideration the real permeability of the membrane



the membrane is represented by specific ion-permeabilities

Electrodiffusion model - transport across the membrane

$$\sum J_k = 0$$

k: Na, K, Cl, ….

$$\Sigma J = J_{K^+} + J_{Na^+} + J_{Cl^-} = 0$$

$$J_k = -D_k\left(\frac{\Delta c_k}{\Delta x} + c_k \frac{z_k F}{RT}\frac{\Delta\varphi}{\Delta x}\right)$$

$$D_k = dp_k$$

$$\varphi_e - \varphi_i = -\frac{RT}{F}\ln\frac{\Sigma p_k^+ c_{ke}^+ + \Sigma p_k^- c_{ki}^-}{\Sigma p_k^+ c_{ki}^+ + \Sigma p_k^- c_{ke}^-}$$

Electrodiffusion model

Goldman – Hodgkin – Katz formula

$$\varphi_e - \varphi_i = -\frac{RT}{F}\ln\frac{\Sigma p_k^+ c_{ke}^+ + \Sigma p_k^- c_{ki}^-}{\Sigma p_k^+ c_{ki}^+ + \Sigma p_k^- c_{ke}^-}$$

c_k: ion-concentration
p_k: permeability constant
e: extracellular
i: intracellular

Electrodiffusion model

Goldman – Hodgkin – Katz formula

$$\varphi_e - \varphi_i = -\frac{RT}{F}\ln\frac{\Sigma p_k^+ c_{ke}^+ + \Sigma p_k^- c_{ki}^-}{\Sigma p_k^+ c_{ki}^+ + \Sigma p_k^- c_{ke}^-}$$

potential (mV)	**Squid axon**	**Rat muscle**
$U_{measured}$	**-62**	**-92**
$\mathbf{U_{GHK}}$	-61,3	-89,2

Good agreement with experimental results

Electrodiffusion model

- Resting U_m depends on the concentration gradients and on the relative permeabilities to Na, K and Cl.

- The GHK equation describes a steady-state condition, not electrochemical equilibrium.

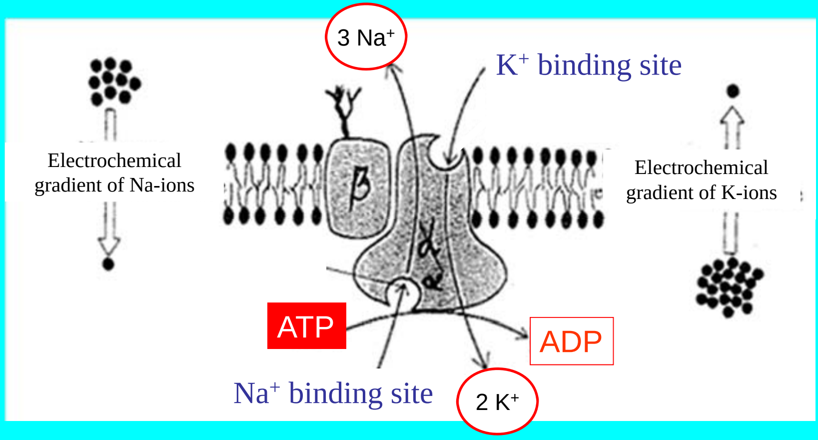
- There is net flux of individual ions, but no net charge movement.

- The cell must supply energy to maintain its ionic gradients.

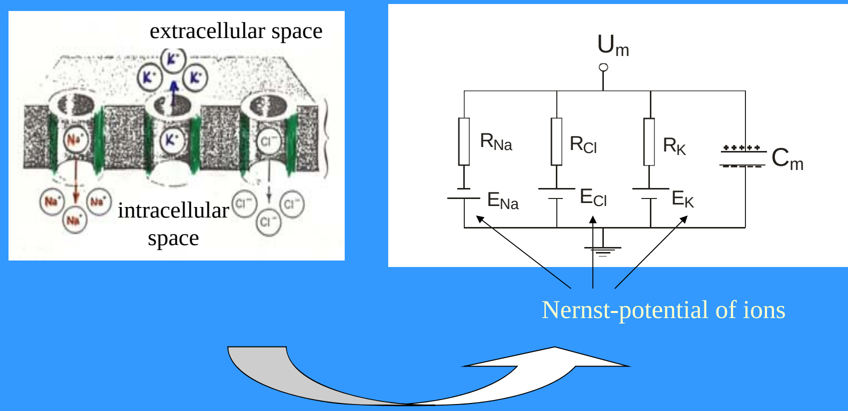
Na - K pump

antiporter

The condition for stationary flow is maintained by the active transport

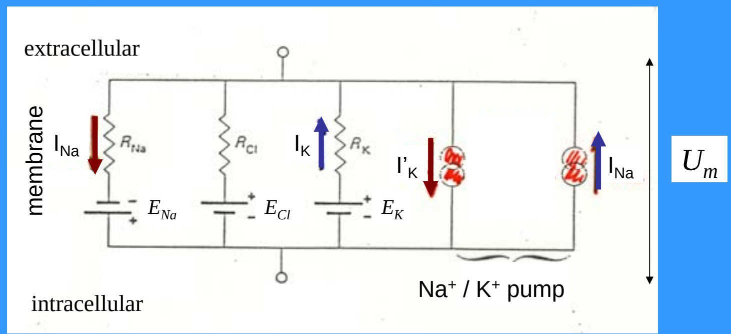


Equivalent circuit model



Ionselective channels modeled by electromotive force and conductivity

Na^+ /K^+ pump restores the ion distribution



Ohm's law: $\boxed{I_k=1/R_k(U_m-E_k)}$

Calculation of resting potential according to the equivalent circuit model

$I_k=1/R_k(U_m-E_k)$

E_k – Nernst-potential of ions

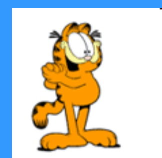
$\Sigma I_k=I_{ion}=0$

$\Sigma I_k=I_{Na}+I_K+I_{Cl}=0$

$g_K (U_m-E_K) + g_{Na} (U_m-E_{Na}) = 0$

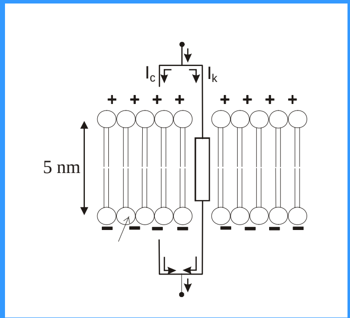
$$U_m = \frac{(U_{0K} x g_K) + (U_{0Na} x g_{Na})}{g_K + g_{Na}}$$

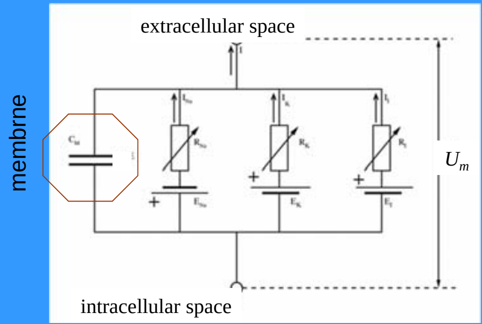
Calculation: $U_m = \frac{(-100 \times 5) + (50 \times 1)}{5+1} = -75$ [mV]



Capacitive property of the membrane

Capacitance ~10^{-6} F/cm^2





$$I_m = I_{ion} + I_c$$

Ion current

Capacitive current

$$I_c = C_m \frac{\Delta U_m}{\Delta t}$$

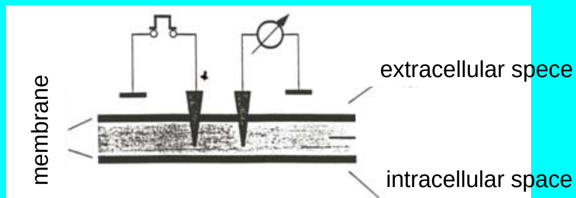
Alteration of resting membrane potential

1. "passive" electric properties of the membrane

Observation

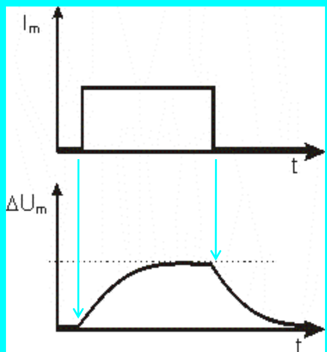
electric current

measuring electrodes



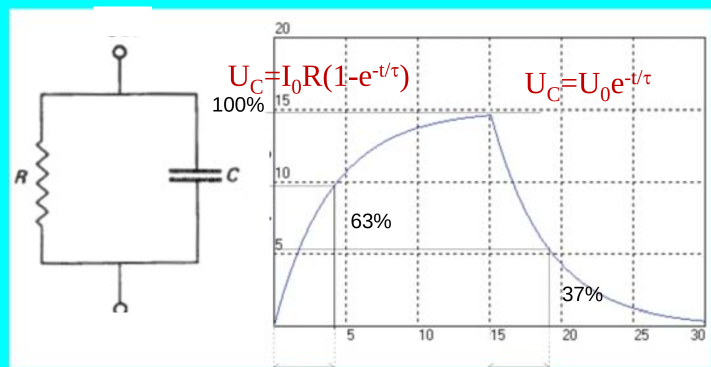
Inward current

Depolarization of the membrane



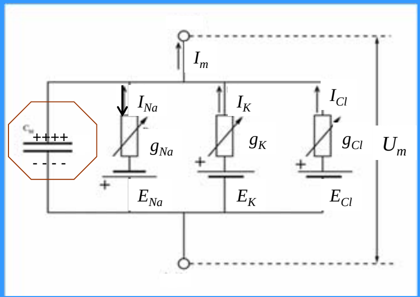
What is it like?

Charge and discharge of RC-circuit



$\tau = RC$

Interpretation with equvivalent circuit model:



$$I_{ion} + I_c = I_m = 0$$

$g_{Na}(U_m - E_{Na}) = I_{Na}$

$g_{ion}(U_m - E) = I_{ion}$

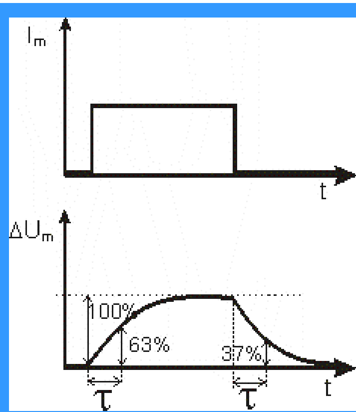
$$C_m \frac{\Delta U_m}{\Delta t} + \frac{\Delta U_m - E}{R_m} - I_{stimulus} = 0$$

Time from the beginning of stimulus

$$\boxed{U_m(t) = U_t\left[1 - e^{-\frac{t}{R_m C_m}}\right]}$$

Membrane potentiel after *t*

Saturation value of membrane potential



Capacitance of the membrane

Resistance of the membrane

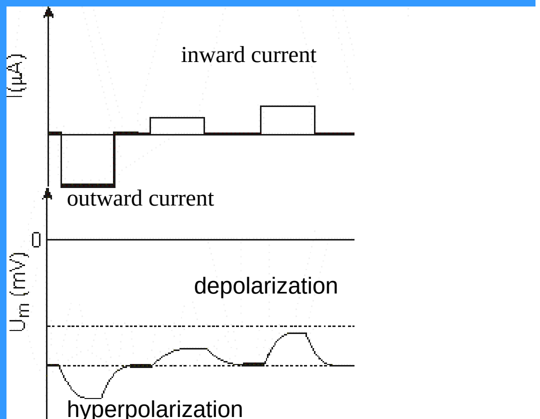
$$\tau = C_m R_m$$

τ : time constant of membrane

-the time required for the membrane potential to reach 63% of its saturation value

-during which the membrane potential decreases to the e-th of its original value

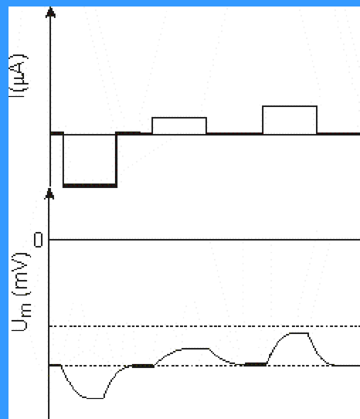
$$U_m(t) = U_t\left[1 - e^{-\frac{t}{R_m C_m}}\right]$$



U_t is proportional to the stimulating current

The rate of the change depends on U_t

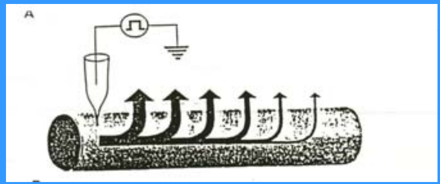
Local changes of membrane potential



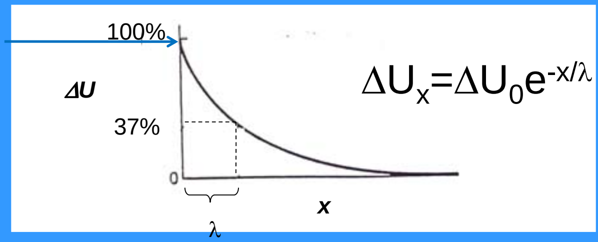
obligate
graded
magnitude varies directly
with the strength of the stimulus
direction varies
with the direction of the stimulus
„localized”

The local changes are not isolated from the neighborhood

Observation



Membrane potential change at the site of stimulation

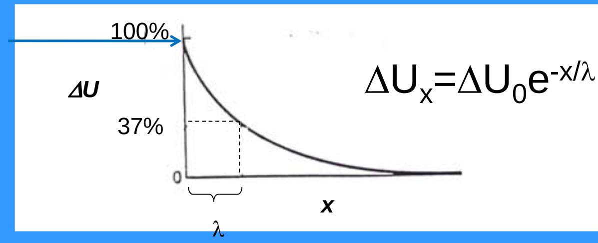


Decrease in amplitude with distance due to leaky membranes

λ: space constant of the membrane:

distance in which the maximal value of induced membrane potential change decreses to its e-th value

Membrane potential change at the site of stimulation



$$\lambda \sim \sqrt{\frac{R_m}{R_i}}$$

Resistance of intracellular space

Local changes of resting membrane potential can be induced

- by electric current pulses
- by adequate stimulus at receptor cells
- by neurotransmitters at postsynaptic membrane
    - excitatory inhibitory postsynaptic potential - depolarization
    - inhibitory postsynaptic potential - hyperpolarization

Significance of the local changes of resting membrane potential

Sensory function

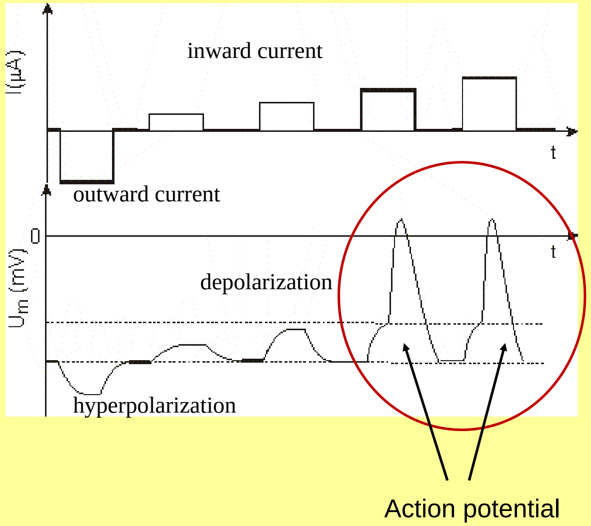
Impulse conduction

Signal transduction

Alteration of resting membrane potential

2. "active" electric properties of the membrane in excited state

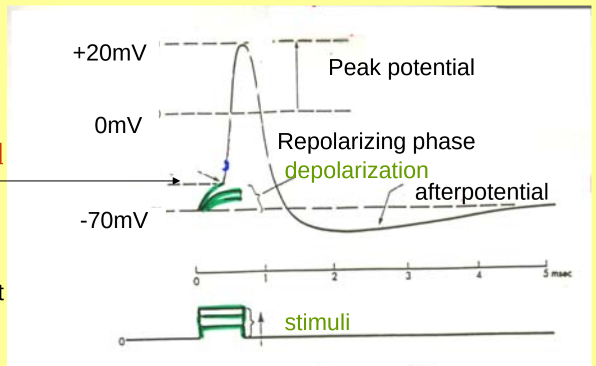
Observation



Phases and landmark of the action potential

Threshold potential

critical membrane potential level at which an action potential can occur



facultative
"All-or-none" amplitude
conducted with constant amplitude

Hodgkin-Katz hypothesis of action potential generation

Voltage-gated, potential sensitive ion channels

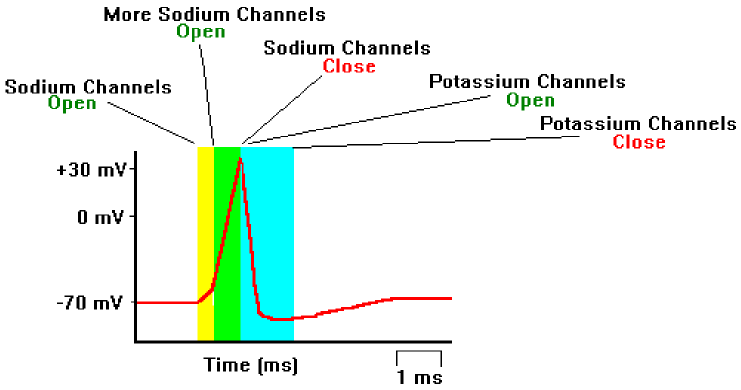
$$\varphi_e - \varphi_i = -\frac{RT}{F}\ln\frac{\Sigma p_k^+ c_{ke}^+ + \Sigma p_k^- c_{ki}^-}{\Sigma p_k^+ c_{ki}^+ + \Sigma p_k^- c_{ke}^-}$$

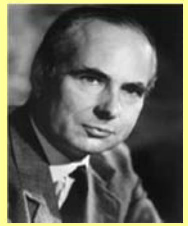
depolarization

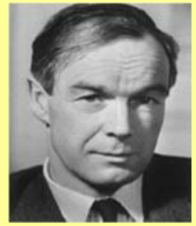
inward Na^+ current

p_{Na} (g_{Na}) increases

Hodgkin-Katz hypothesis of action potential sequence







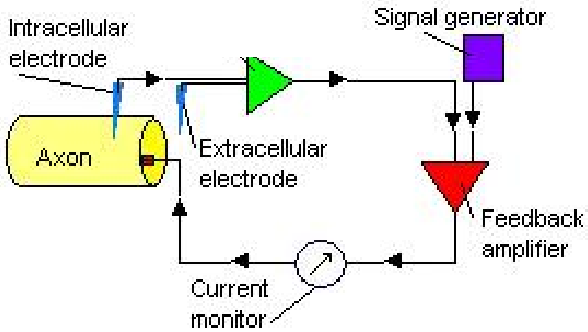
Andrew Fielding Huxley (1917-)

Alan Loyd Hodgkin (1914-1998)

The Nobel Prize in Physiology or Medicine 1963

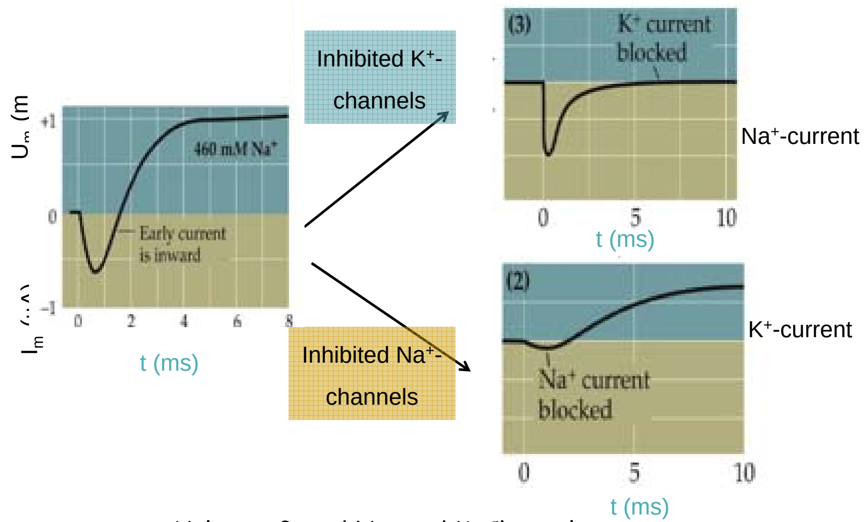
"for their discoveries concerning the ionic mechanisms involved in excitation and inhibition in the peripheral and central portions of the nerve cell membrane"

Voltage Clamp



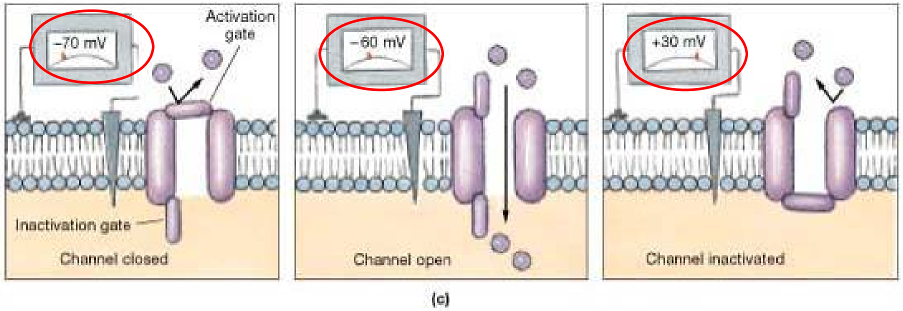
- Membrane potential is stabilized
- Ionic current is measured

Measurement of separated ionic currents



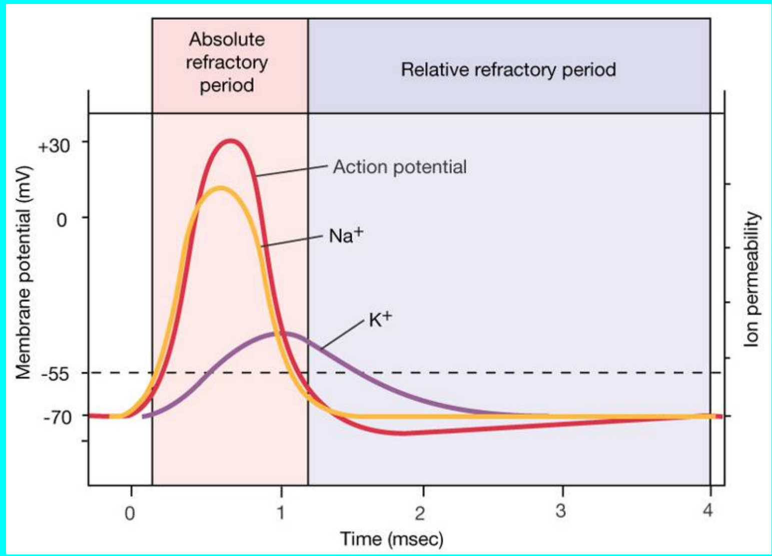
Voltage-Gated Na^+ and K^+ Channels

States of voltage-gated sodium channels



at depolarization threshold

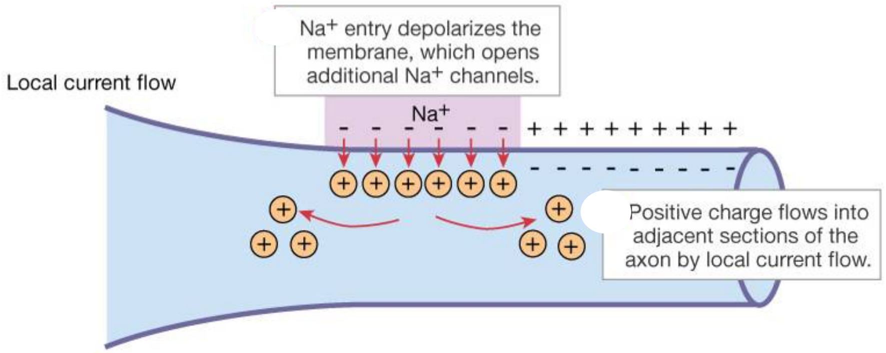
Conductivities during action potential



Factors Influencing Conduction Direction and Velocity

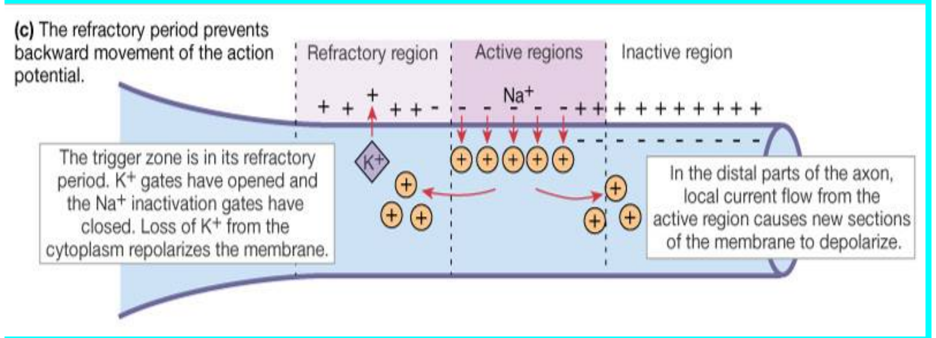
The evolutionary need for the fast and efficient transduction of electrical signals

Propagation of action potential (1)



based on local current flow and depolarization of adjacent membrane area

Propagation of action potential (2)



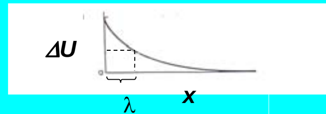
Speed and distance of propagation?

How are the ***time constant*** and the ***space constant*** related to propagation velocity of action potentials

---

Generation of the next peak potential

Where?



The greater the space constant, the more rapidly distant regions will be brought to threshold and the more rapid will be the propagation velocity

When?

The smaller the time constant, the more rapidly a depolarization will affect the adjacent region.

Velocity is the function of passive properties – τ and λ – of membranes

---

Effect of axon diameter:

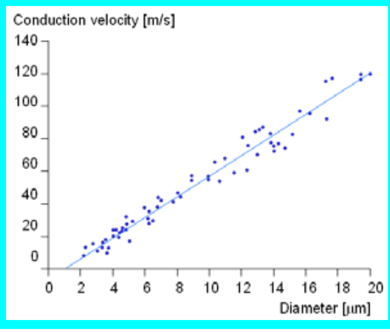
$$\tau = C_m R_m$$

$$\lambda \sim \sqrt{\frac{R_m}{R_i}}$$

r↑ → R_i↓ (~1/r²), R_m↓ (~1/r) → τ↓, λ↑

Squid giant axon r=250μm v=25m/s

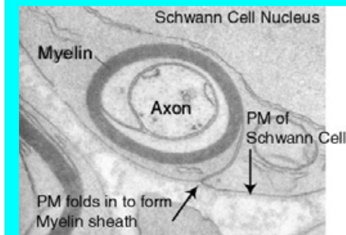
human nerve cell r= 10 μm v≠0.5m/s ?

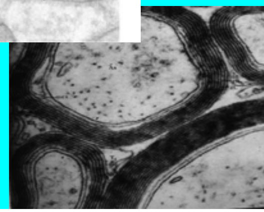


Myelination!

R_m – very high → big space constant

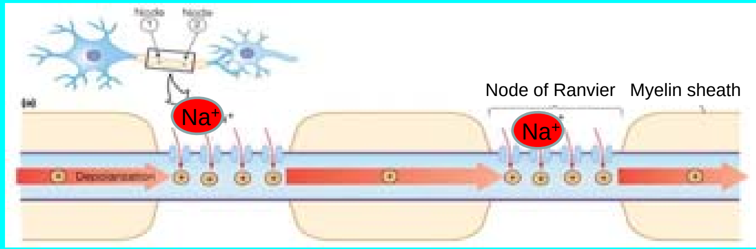
C_m – very small → small time constant



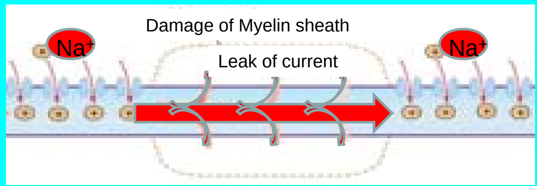


human nerve cell r= 10 μm v~ 100 m/s

Saltatory conduction – quick, energy saving



Myelin prevents ions from entering or leaving the axon along myelinated segments.



Effect of axon diameter and Myelination

The diameter of frog axons and the presence or absence of myelination control the conduction velocity.

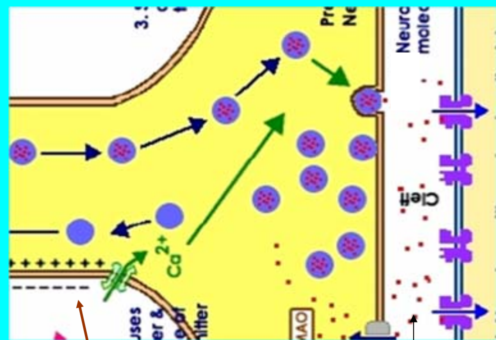
Fiber type	Average axon diameter (μm)	Conduction velocity ($m \cdot s^{-1}$)
Myelinated fibers		
Aα	18.5	42
Aβ	14.0	25
Aγ	11.0	17
B	Approximately 3.0	4.2
Unmyelinated fibers		
C	2.5	0.4–0.5

Effect of passive electric properties on signal transduction in synapses

Signal transmission in synapses

presynaptic terminal

postynaptic terminal



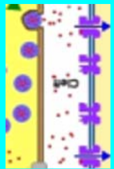
Action potential

neurotransmitter

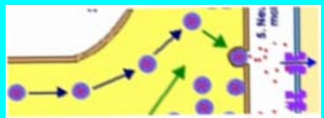
How can neurons transmit information from presynaptic to

postsynaptic cells **if most synaptic effects are subthreshold**?

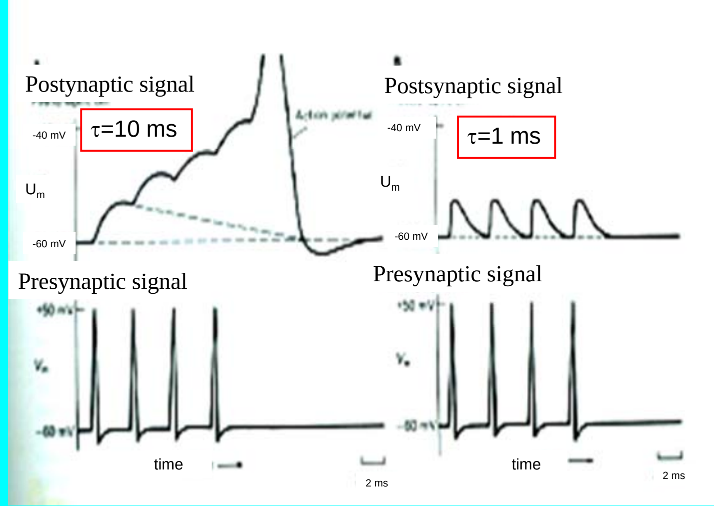
Spatial Summation : combined influences at the same cell at a particular moment in time



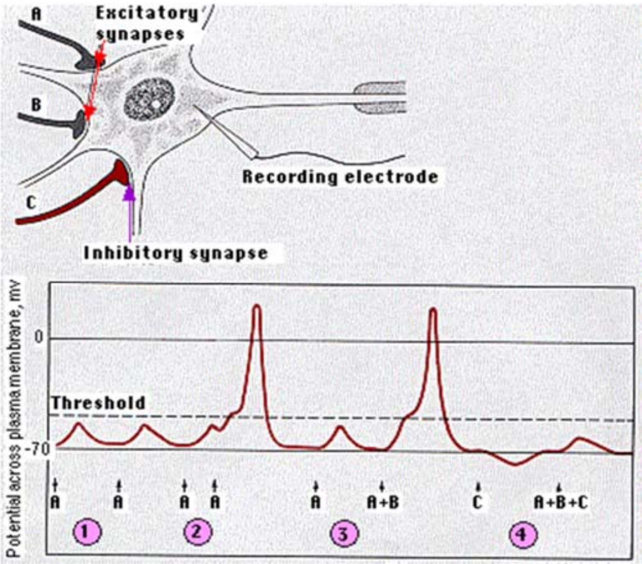
Temporal Summation : combined effects of neurotransmitter release from the same sites over time



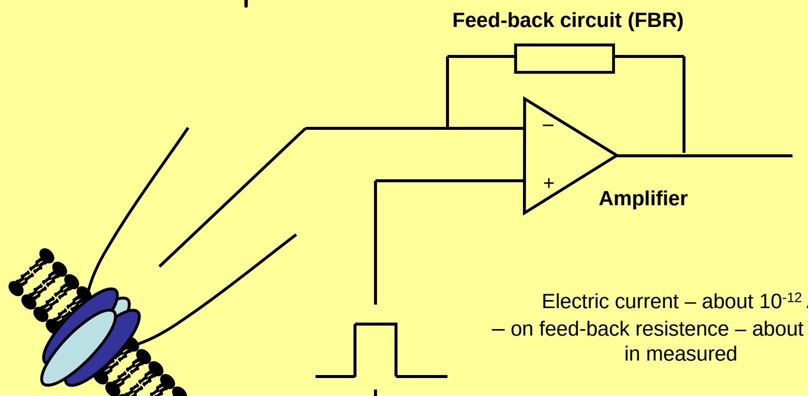
Temporal Summation : combined effects of neurotransmitter release from the same sites over time



Temporal and spatial summation

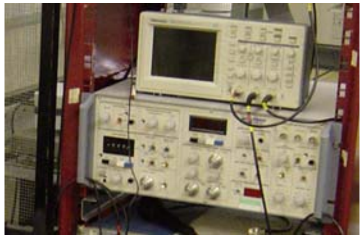


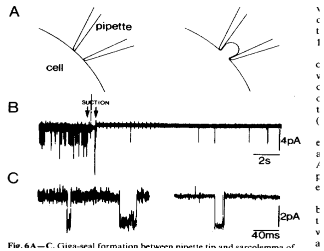
Patch-Clamp circuit



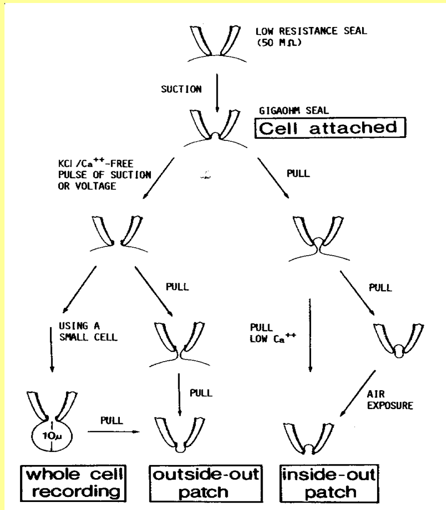
Electric current – about 10^{-12} A
– on feed-back resistence – about 50 GΩ
in measured

Patch-Clamp technique



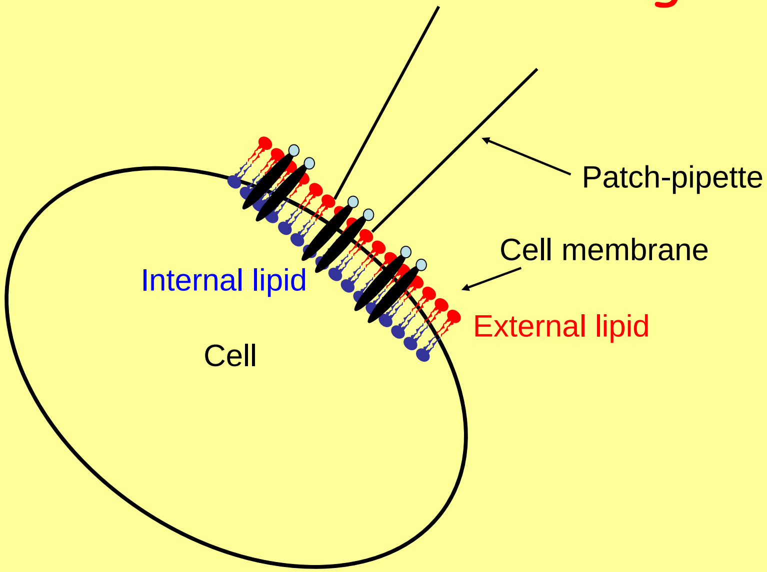


Patch-Clamp variations

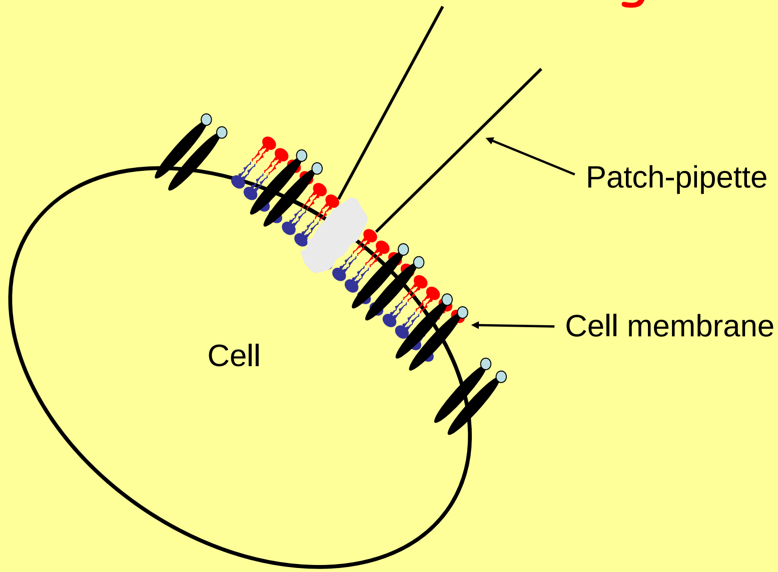


From Hamill *et al* 1981

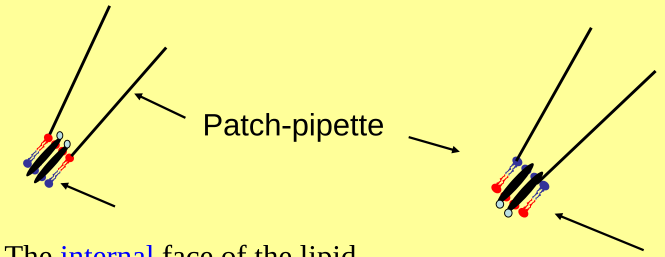
Cell-attached recording



Whole-cell recording



Inside-out recording

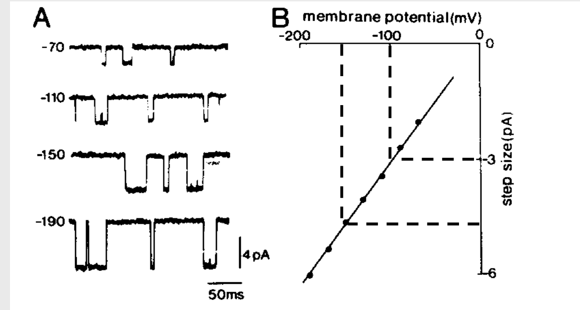


The internal face of the lipid bi-layer faces the bath solution

The external face of the lipid bi-layer faces the bath solution

Outside-out recording

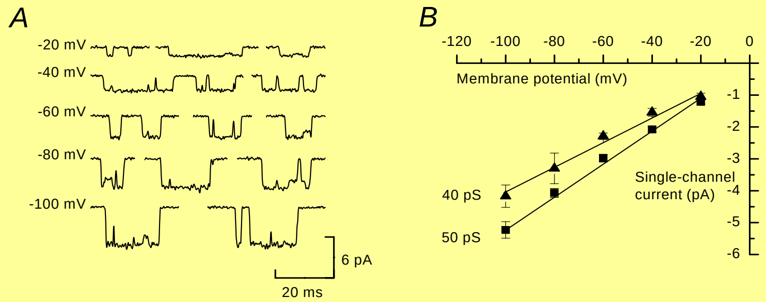
Single-channel I/V plots are used to determine the conductance of an ion channel



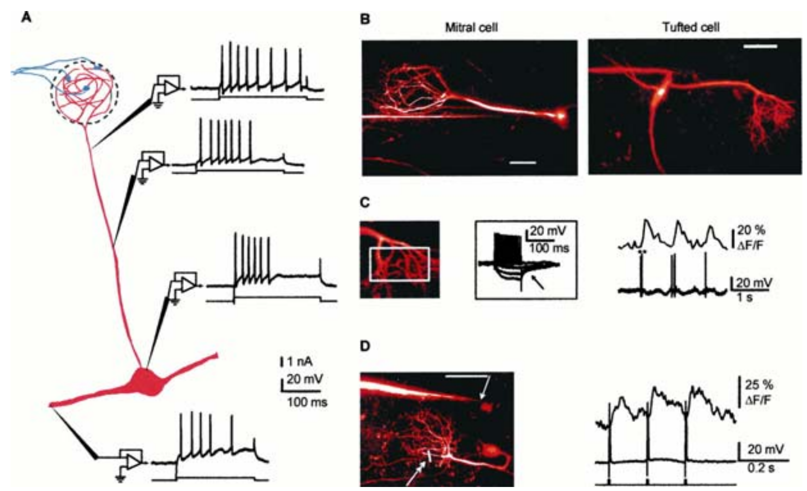
$$g_{channel} = \Delta I \div \Delta V$$
$$= 1.6 \times 10^{-12}\,A \div 50 \times 10^{-3}\,V$$
$$= 32 \times 10^{-12}\,S$$
$$= 32\,pS$$

From Hamill *et al* 1981

Single channel with multiple states



Sodium action potentials synchronize [Ca2+] transients in all dendritic compartments of mitral cells in the olfactory bulb of anesthetized rats.



Charpak S et al. PNAS 2001;98:1230-1234