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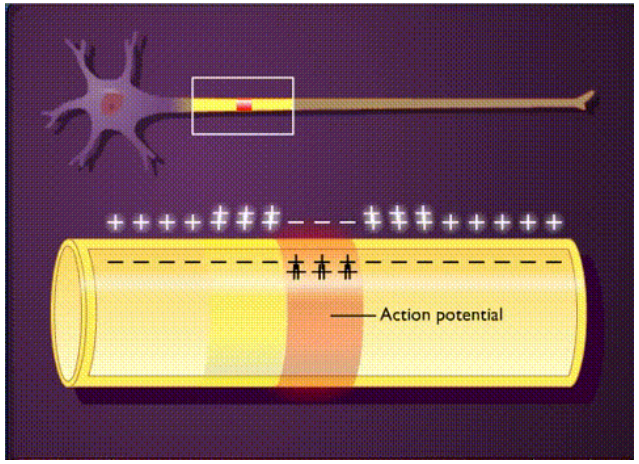


Lectures on Medical Biophysics

Biological membranes and bioelectric phenomena

A part of this lecture was prepared on the basis of a presentation kindly provided by Prof. **Katarína Kozlíková** from the Dept. of Medical Biophysics, Medical Faculty, Comenius University in Bratislava

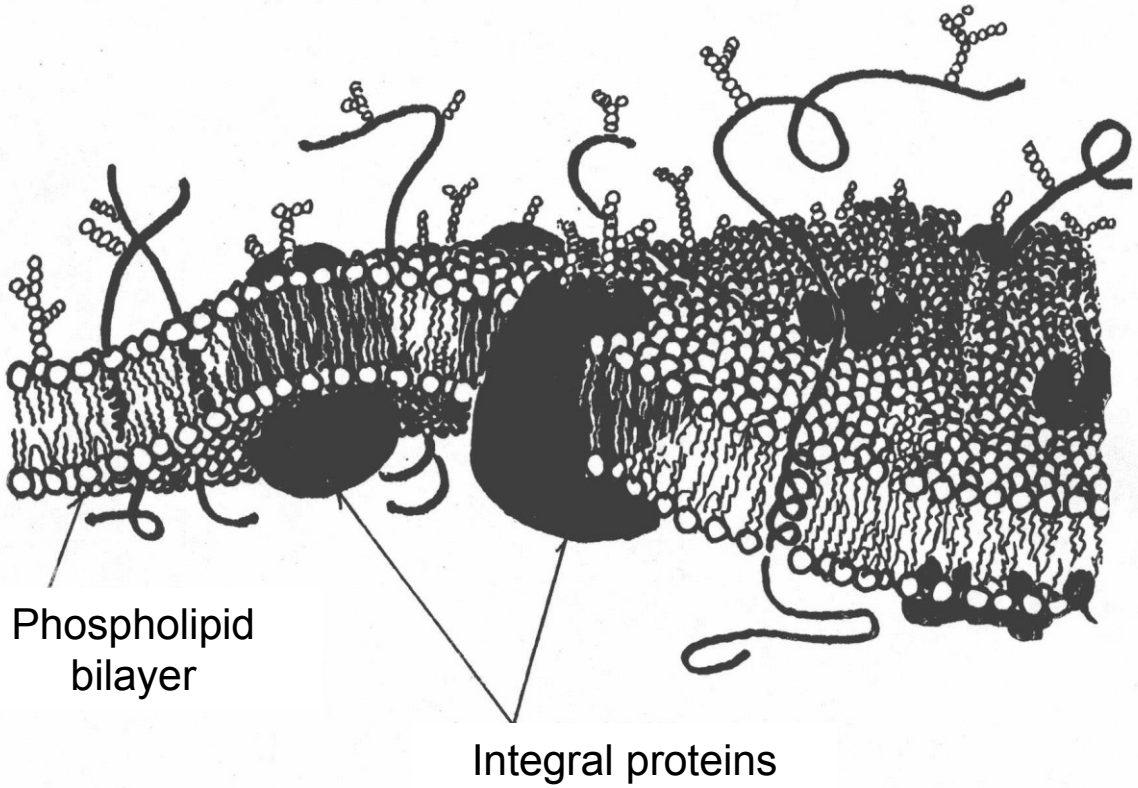
Bioelectric phenomena

- The electric signal play a key role in controlling of all vitally important organs. They ensure fast transmission of information in the organism. They propagate through nerve fibres and muscle cells where they trigger a chain of events resulting in muscle contraction. They take a part in basic function mechanisms of sensory and other body organs.
- On cellular level, they originate in membrane systems, and their propagation is accompanied by production of electromagnetic field in the ambient medium.
- Recording of electrical or magnetic signals from the body surface is fundamental in many important clinical diagnostic methods.

Biological membrane

- It is not possible to understand the origin of resting and action membrane voltage (potential) without knowledge of structure and properties of biological membrane.
- In principle, it is an electrically non-conducting thin bilayer (6-8 nm) of phospholipid molecules. There are also built-in macromolecules of proteins with various functions. Considering electrical phenomena, two kinds of proteins are the most important: the ion *channels* and *pumps*. In both cases these are components of transport mechanisms allowing transport of ions through the non-conducting phospholipid membrane.

Structure of the membrane



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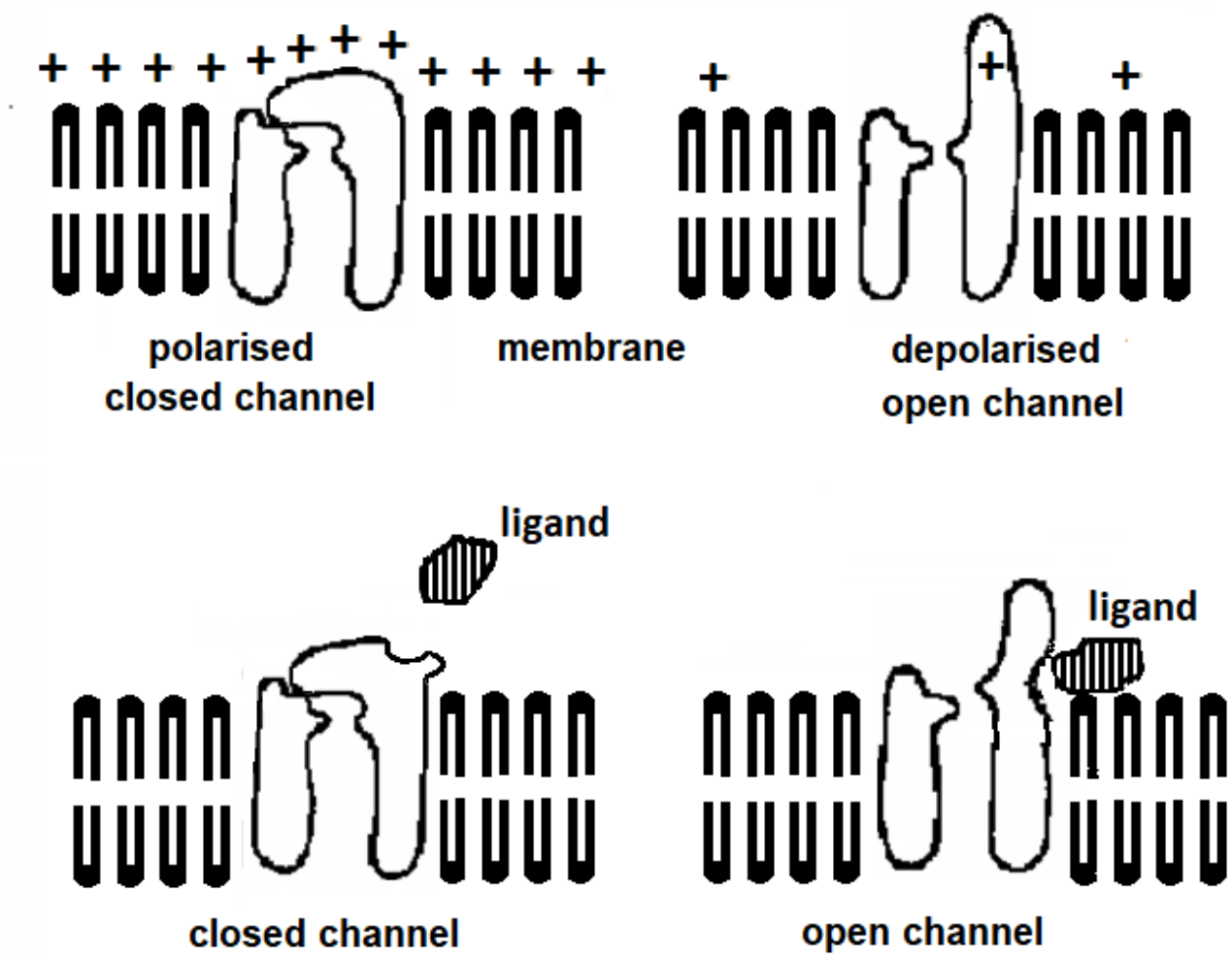
Channels

- The basic mechanism of the ion exchange between internal and external medium of the cell are the membrane channels. They are protein molecules but, contrary to the pumps with stable binding sites for the transmitted ions, they form water-permeable pores in the membrane. Opening and closing of the channels (*gating*) is performed in several ways. Besides the electrical gating we can encounter gating controlled by other stimuli in some channels (chemical binding of substances, mechanical tension etc.).
- The passage of ions through the channel cannot be considered to be free diffusion because most channels are characterised by certain selectivity in ion permeability. Sodium, potassium, calcium or chloride channels are distinguished.
- In this kind of ion transport there is no need of energy delivery.

Electrical and chemical gating



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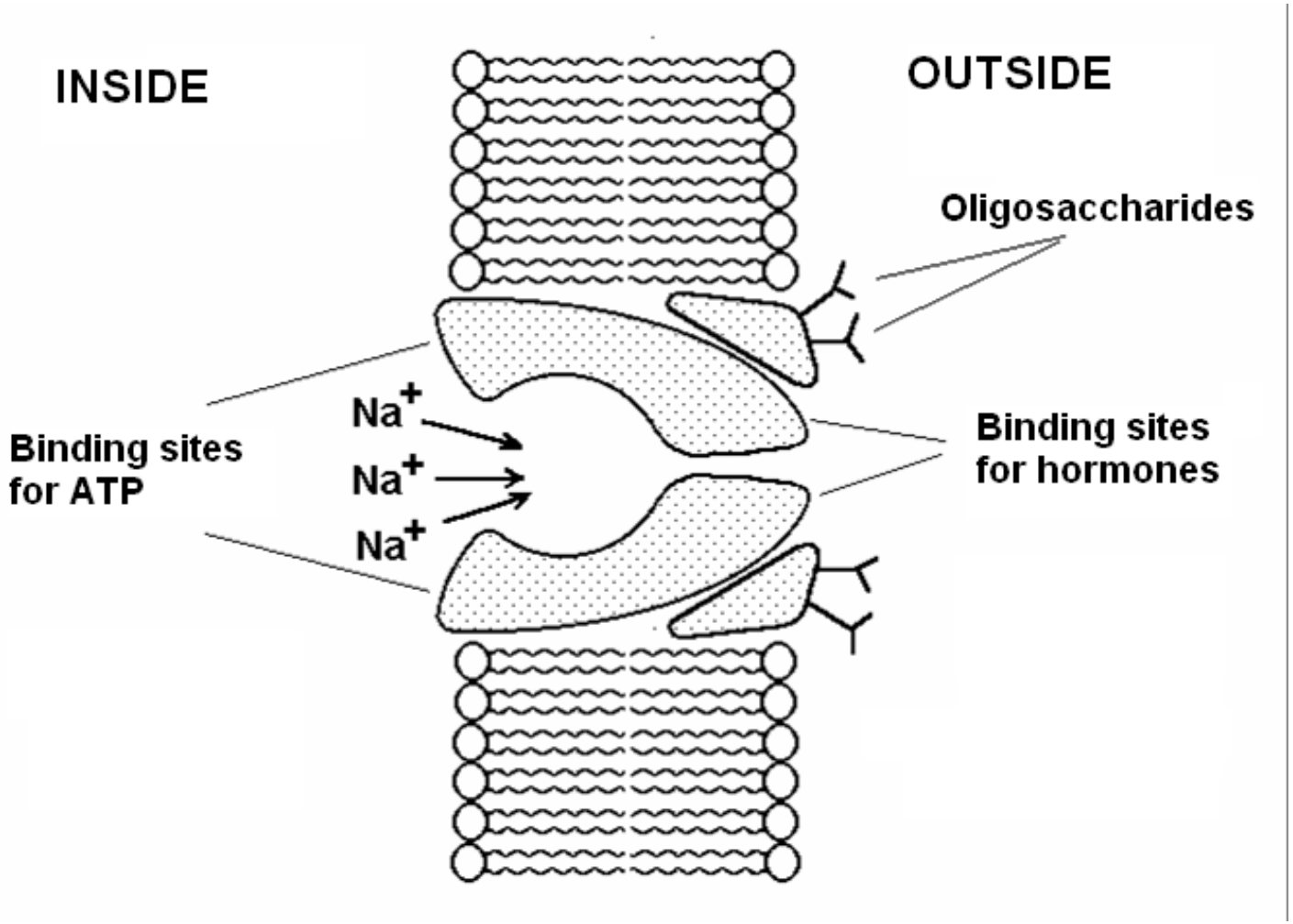
Ion transport systems

Many ion transport systems were discovered in cell membranes. One of them, denoted as *sodium-potassium pump (Na/K pump* or Na^+-K^+-*ATP-ase)* has an extraordinary importance for production of membrane voltage. It removes *Na*-ions from the cell and interchanges them with *K*-ions. Thus, the concentrations of these ions in the intracellular and extracellular medium (they are denoted as *[Na+], [K+]* and distinguished by indexes *i*, *e*) are different. We can write:

Working *Na/K* pump requires constant energy supply. This energy is delivered to the transport molecules by the adenosine triphosphate (ATP) which is present in the intracellular medium.

Principle of the sodium-potassium pump



The sodium ions are released on the outer side of the membrane. Following conformation change of the ion pump molecule enables binding of potassium ions which are carried inside the cell.

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Function of biological membranes

- They form the interface between the cells and also between cell compartments.
- They keep constant chemical composition inside bounded areas by selective transport mechanisms.
- They are medium for fast biochemical turnover done by enzyme systems.
- Their specific structure and selective ion permeability is a basis of bioelectric phenomena.

Excitability

Characteristic feature of living systems on any level of organisation of living matter

An important condition of adaptation of living organisms to environment

An extraordinary ability of some specialised cells (or tissues – muscle cells, nerve cells)

Each kind of excitable tissue responses most easily on a certain energetic impulse (the *adequate stimulus*). Another energetic impulse can also evoke an excitation but much more energy is necessary (the inadequate stimulus).

Resting membrane potential

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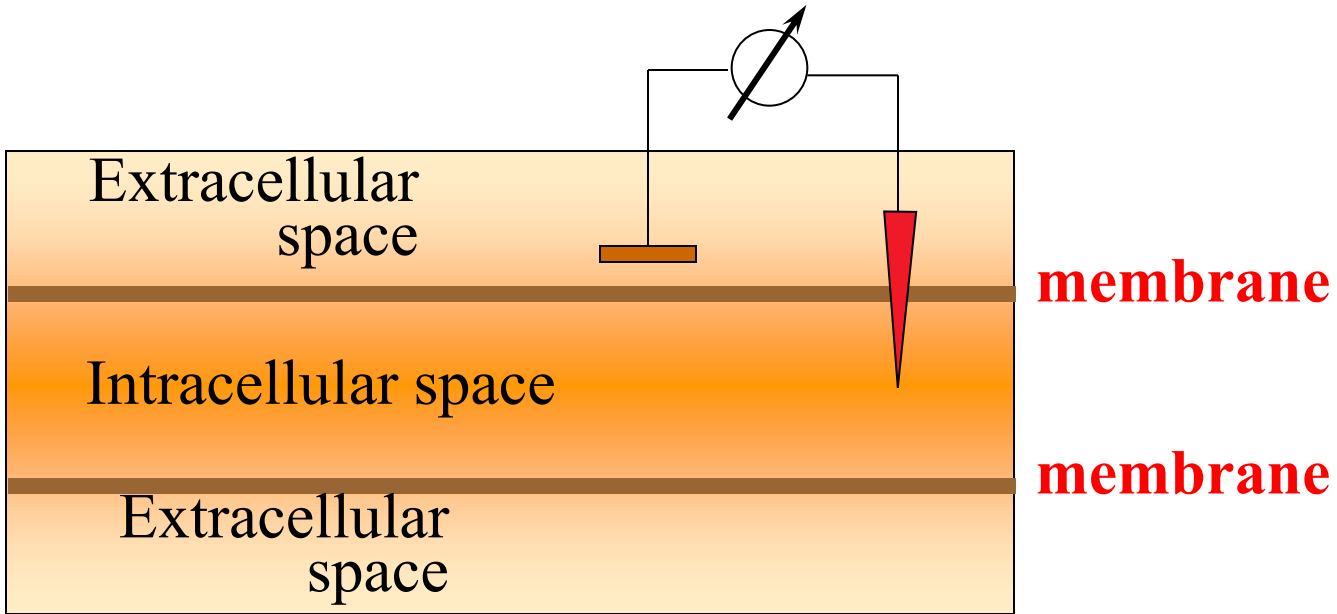
Resting membrane potential – RMP (1)

Potential difference between a microelectrode inside the cell *(negative potential)* and a surface electrode outside the cell *(zero potential)* = **membrane voltage = membrane potential**

„Non-polarisable“ electrodes are used



MUNI Resting membrane potential – RMP (2)

Its values depend on:
- Type of the cell
- Art of the animal the cell is taken from
- For identical cells – on the composition and concentration of the ion components of the extracellular liquids

The value of RMP at normal ion composition of the IC and EC liquid: -100 mV to -50 mV

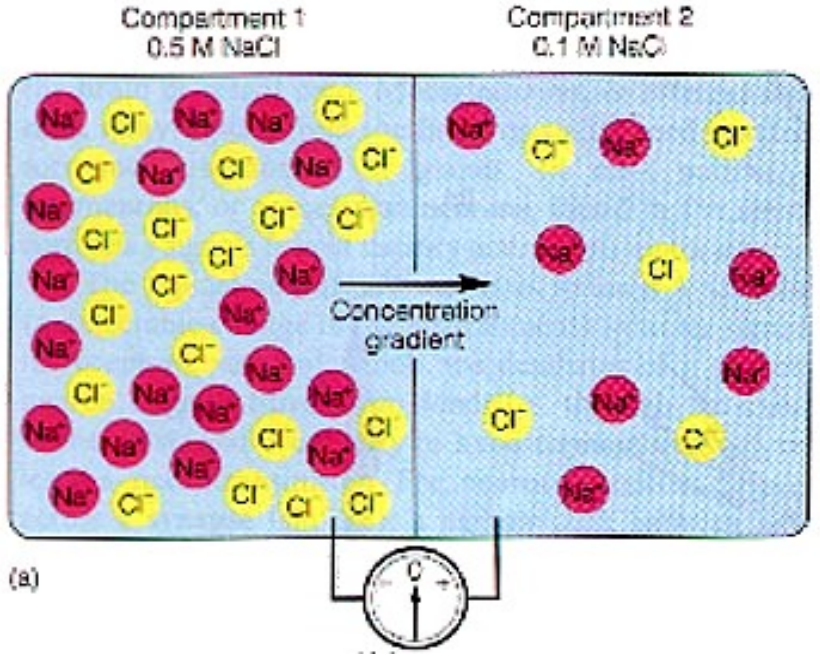
Membrane thickness ~ 10 nm

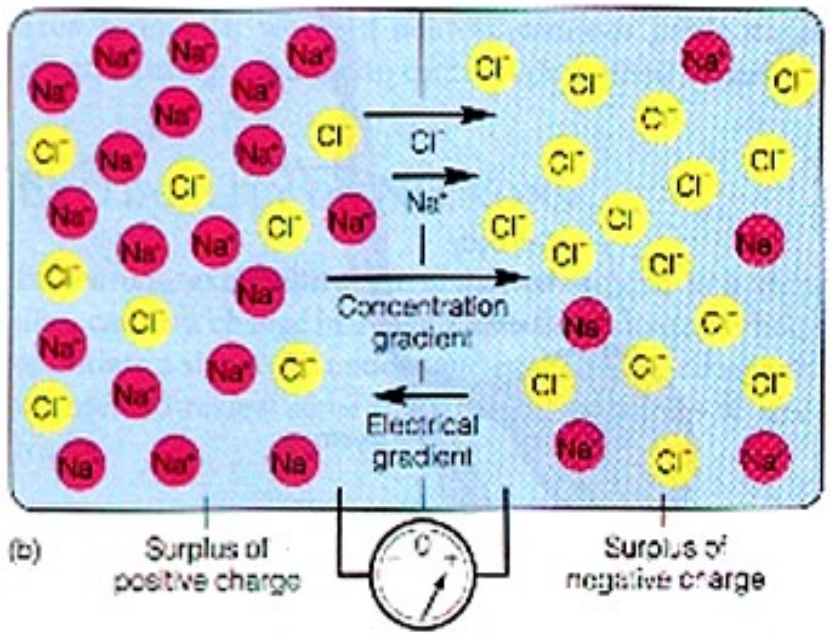
Result: Electric field intensity in the membrane ~ 10^7 V/m

To compare: Electric field intensity on the Earth's surface ~ 10^2 V/m

Diffusion potential DP (1)

Caused by diffusion of charged particles DP in non-living systems – solutions are separated by a membrane permeable for Na^+ and Cl^-.





Electric field repulses Cl^- from [2]

The compartments are electroneutral, but there is a concentration gradient

⇒ Diffusion of ions from [1] do [2]

Hydration envelope (water molecules are bound to ions) **Na^+** (more) **a Cl^-** (less)

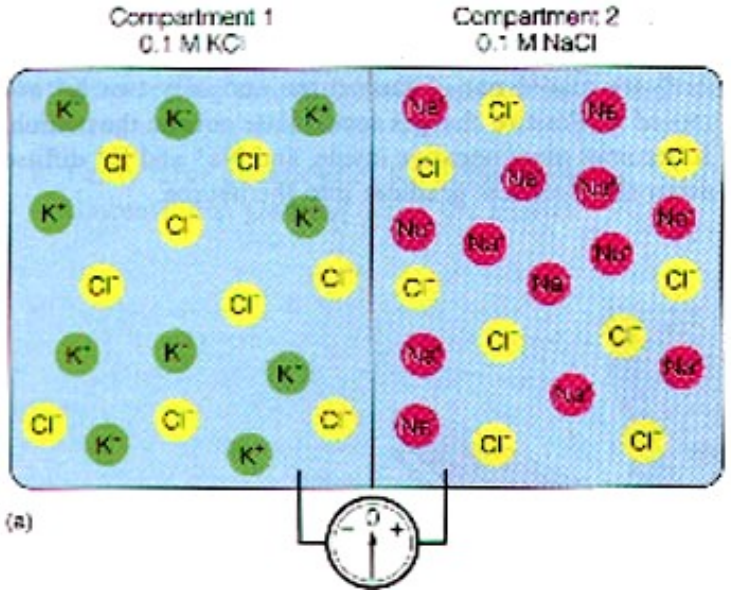
⇒ faster diffusion of Cl^- against (!) concentration gradient

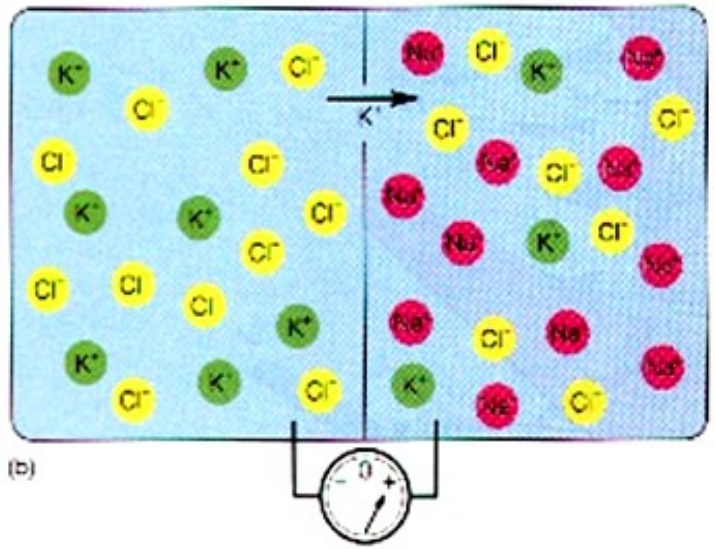
⇒ **Transient** voltage appears across the two compartments

⇒ Diffusion potential

Diffusion potential DP (2)

DP in living systems – the solutions are separated by a **selectively permeable membrane for K^+, n**on-permeable for pro Na^+ a Cl^-**.**





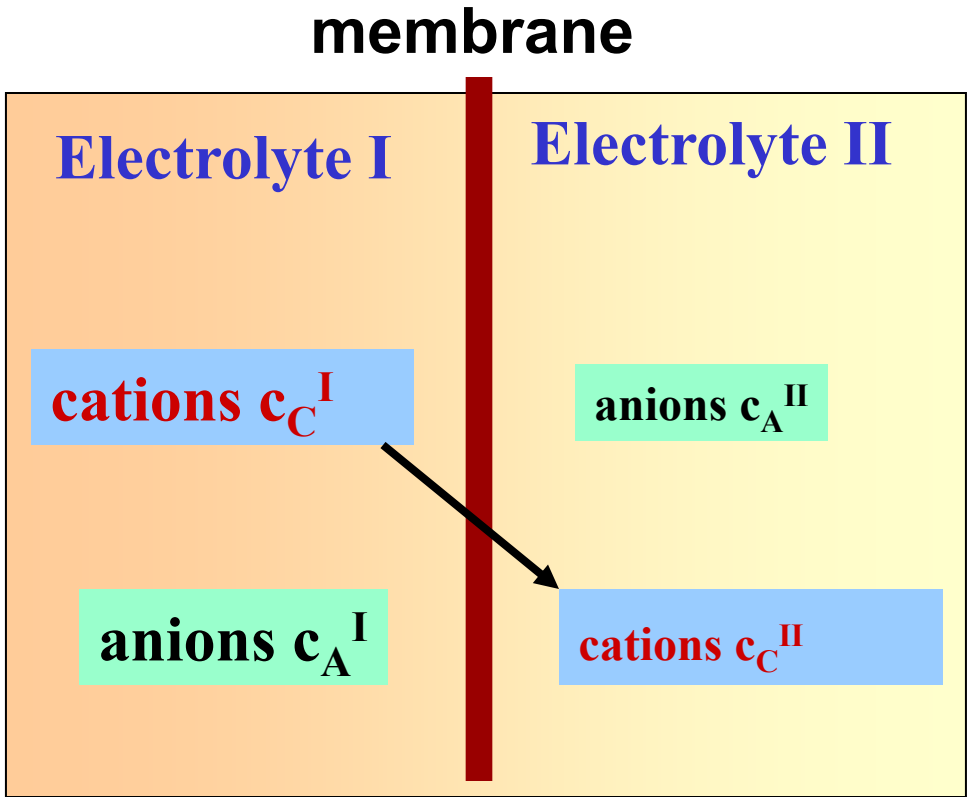
In such a system, an equilibrium arises if there is no resulting flux of ions.

⇒ **Diffusion of K^+ against its concentration gradient occurs until an electric gradient of the same magnitude, but of opposite direction arises**

⇒ **An equilibrium potential emerges – resulting diffusion flux is equal to zero**

A simple example of a membrane equilibrium (1)

The same electrolyte is on both sides of the membrane but of different concentrations ($c_I > c_{II}$), **the membrane is permeable only for cations**



Result:

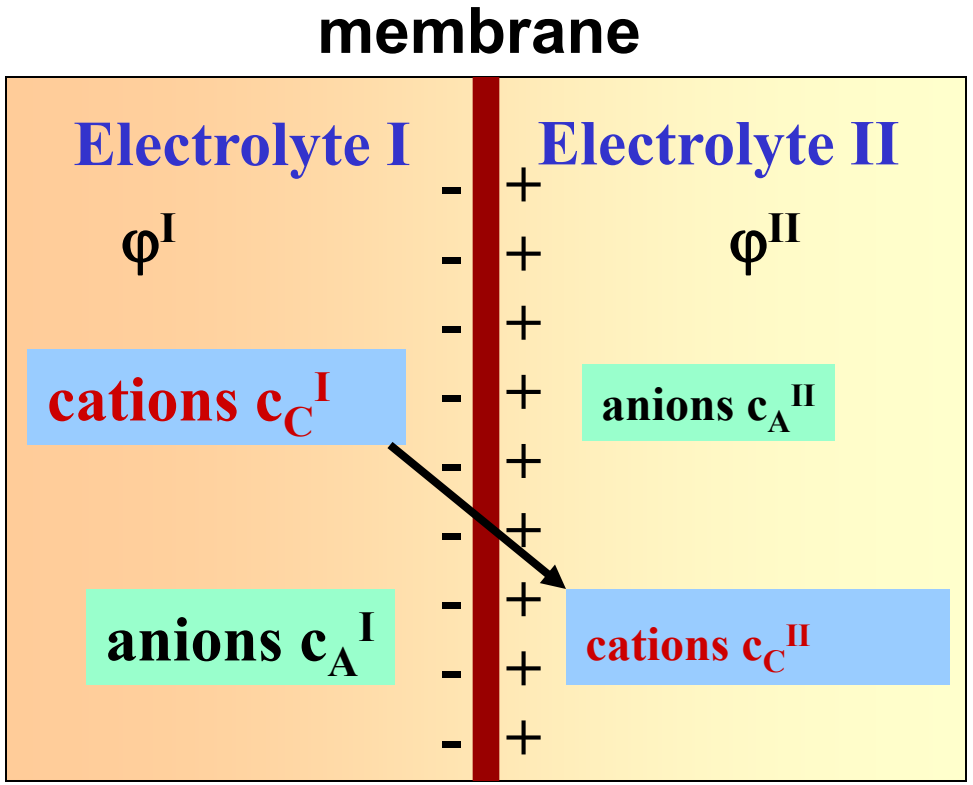
Electric double layer is formed on the membrane

layer 1: anions stopped in space I

layer 2: cations attracted to the anions (II)

A simple example of a membrane equilibrium (2)

The concentration difference ”drives” the cations, electric field of the bilayer “pushes them back”



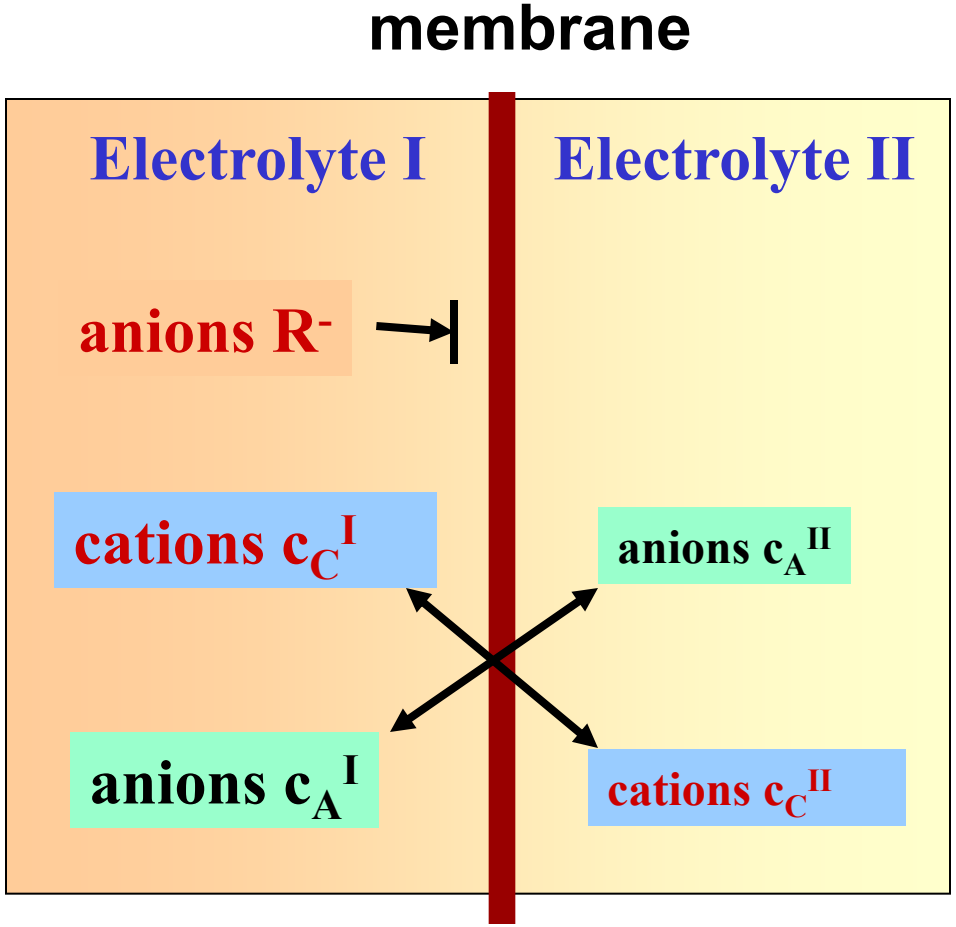
In equilibrium: potential difference *U* arises:

$$U = \varphi^{II} - \varphi^{I} = -\frac{RT}{zF} ln \frac{c_K^I}{c_K^{II}}$$

(Nernst equation)

M U N I Donnan equilibrium (1)

The same electrolyte is on both sides, concentrations are different ($c^{I} > c^{II}$), **membrane is permeable for small univalent ions C^+ and A^-, non-permeable for R-** .



Diffusible ions: C^+, A^- diffuse freely
non-diffusible ions: R^- i.e. big anions

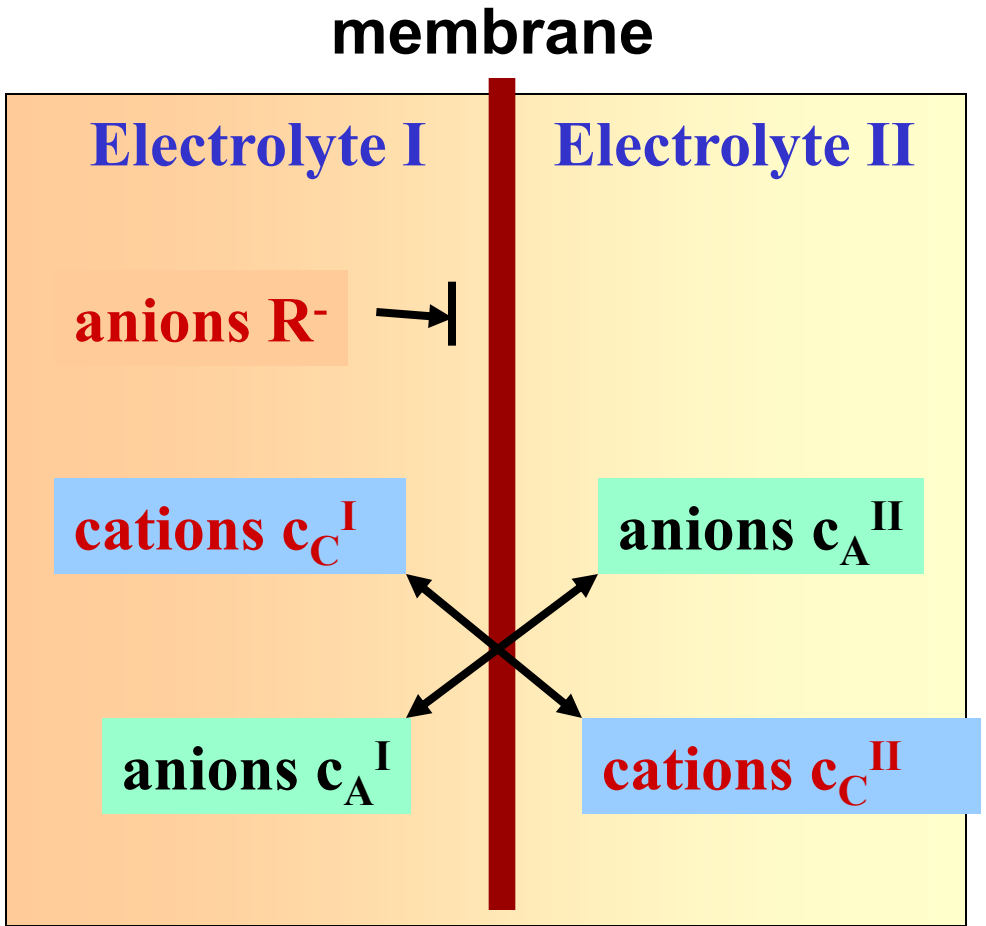
In presence of R^-: Equal distribution of C^+ and A^- cannot be achieved

$\Rightarrow$ a special case of equilibrium - **Donnan equilibrium**

Donnan equilibrium (2)

Equilibrium concentrations:

$$c_K^I c_A^I = c_K^{II} c_A^{II}$$



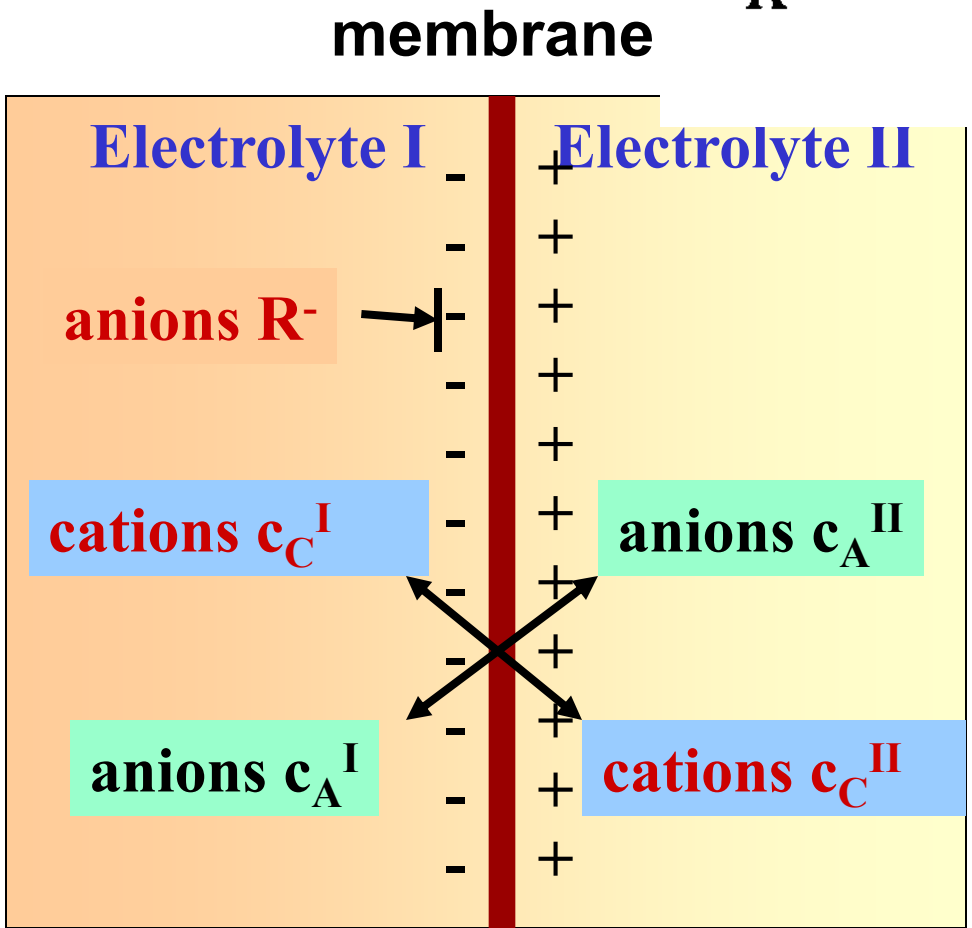
Donnan ratio:

$$\frac{c_K^I}{c_K^{II}} = \frac{c_A^{II}}{c_A^I} = r$$

MUNI Donnan equilibrium (3)

Donnan ratio:

$$\frac{c_K^I}{c_K^{II}} = \frac{c_A^{II}}{c_A^I} = r \qquad r = \sqrt[z_i]{\frac{c_i^I}{c_i^{II}}}$$



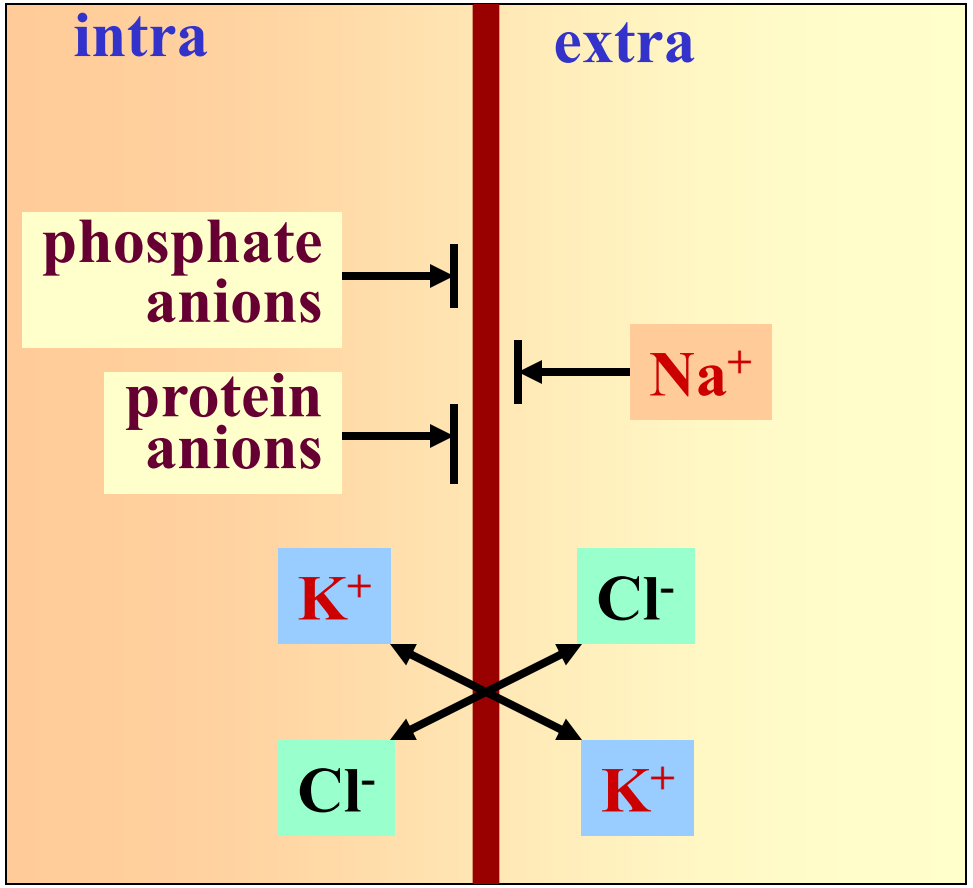
Donnan potential:

$$U = \varphi^{II} - \varphi^I = -\frac{RT}{F} ln \frac{c_K^I}{c_K^{II}}$$

$$= -\frac{RT}{F} ln \frac{c_A^{II}}{c_A^I} = -\frac{RT}{F} lnr$$

Donnan model in living cell (1)

cell membrane



diffuse: K+, Cl-

do not diffuse: Na+, anions, also proteins and nucleic acids

Concentrations:

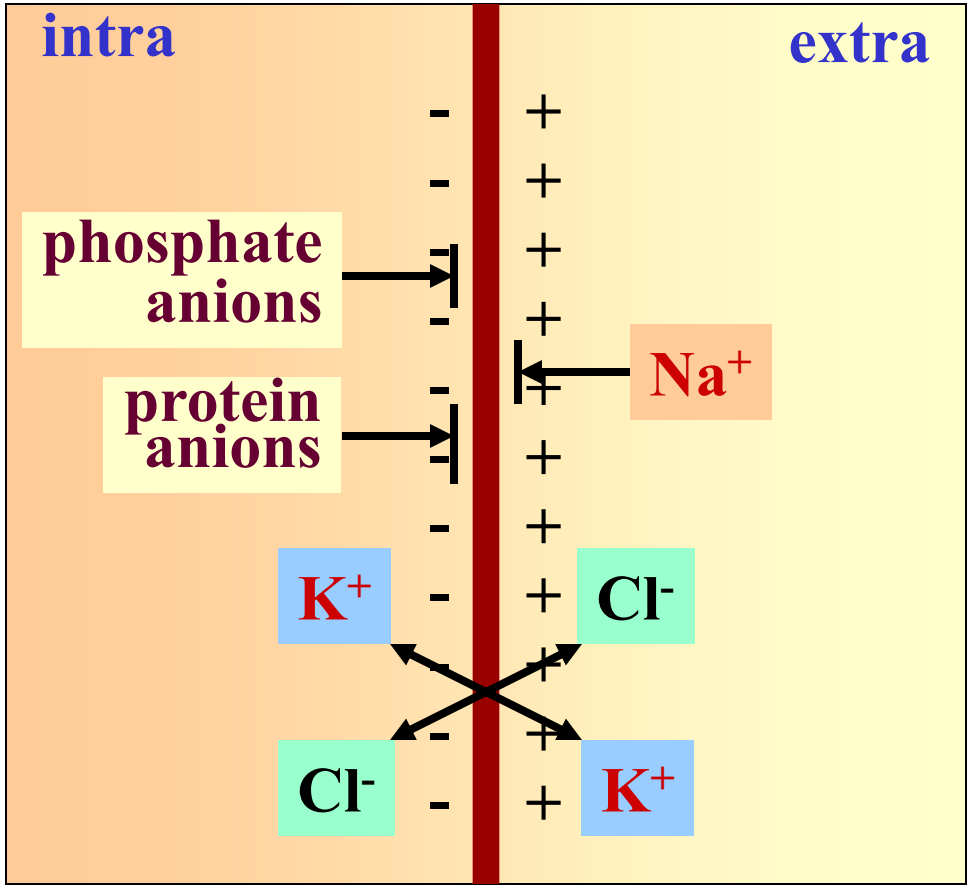
$[K^+]_{in} > [K^+]_{ex}$

$[Cl^-]_{in} < [Cl^-]_{ex}$

Donnan model in living cell (2)

Donnan ratio:

Cell membrane



Donnan potential:

MUNI Donnan model in living cell (3)

Donnan potential (resting potential) [mV]:

object:	calculated:		measured:
	K^+:	Cl^-:	
cuttlefish axon	- 91	- 103	- 62
frog muscle	- 56	- 59	- 92
rat muscle	- 95	- 86	- 92

Donnan model differs from reality:

The cell and its surroundings are regarded as closed thermodynamic systems

The diffusible ions are regarded as fully diffusible, the membrane is no barrier for the diffusible ions

The effect of ionic pumps on the concentration of ions is neglected

The interaction between membrane and ions is not considered

Model of ion transport (1)

Electrodiffusion model with smaller number of simplifications

We suppose:

A constant concentration difference between outer and inner side of the membrane ⇒ constant transport rate through membrane

Migration of ions through membrane ⇒ electric bilayer on both sides of the membrane

All kinds of ions on the both sides of the membrane are considered simultaneously

Empirical fact – **different ions have different non-zero permeability**

Model of ion transport (2)

Goldman - Hodgkin - Katz

$$U = \frac{RT}{F} \ln \frac{\sum P_{ki} c_{ki\ EXT} + \sum P_{ai} c_{ai\ INT}}{\sum P_{ki} c_{ki\ INT} + \sum P_{ai} c_{ai\ EXT}}$$

k = cations, a = anions

$$U = \frac{2.3RT}{F} \log \frac{P_{K+}\left[K^+\right]_{EXT} + P_{Na+}\left[Na^+\right]_{EXT} + P_{Cl-}\left[Cl^-\right]_{INT}}{P_{K+}\left[K^+\right]_{INT} + P_{Na+}\left[Na^+\right]_{INT} + P_{Cl-}\left[Cl^-\right]_{EXT}}$$

P - permeability

Model of ion transport (3)

„giant“ cuttlefish axon (t = 25°C):

$$p_K : p_{Na} : p_{Cl} = 1 : 0.04 : 0.45$$

calculated: U = - 61 mV

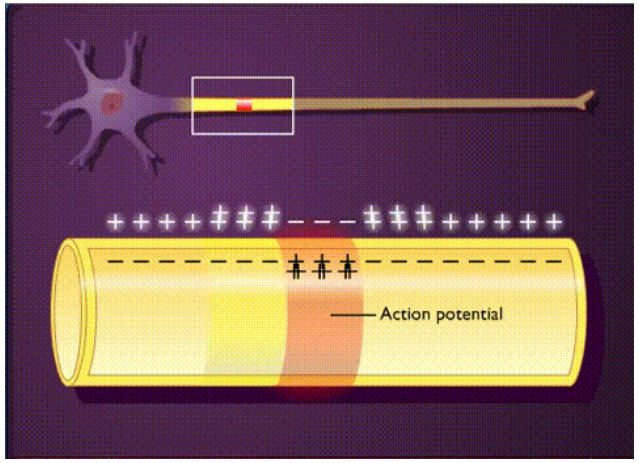
measured: U = - 62 mV

frog muscle (t = 25°C):

$$p_K : p_{Na} : p_{Cl} = 1 : 0.01 : 2$$

calculated: U = - 90 mV

measured: U = - 92 mV



Action potential

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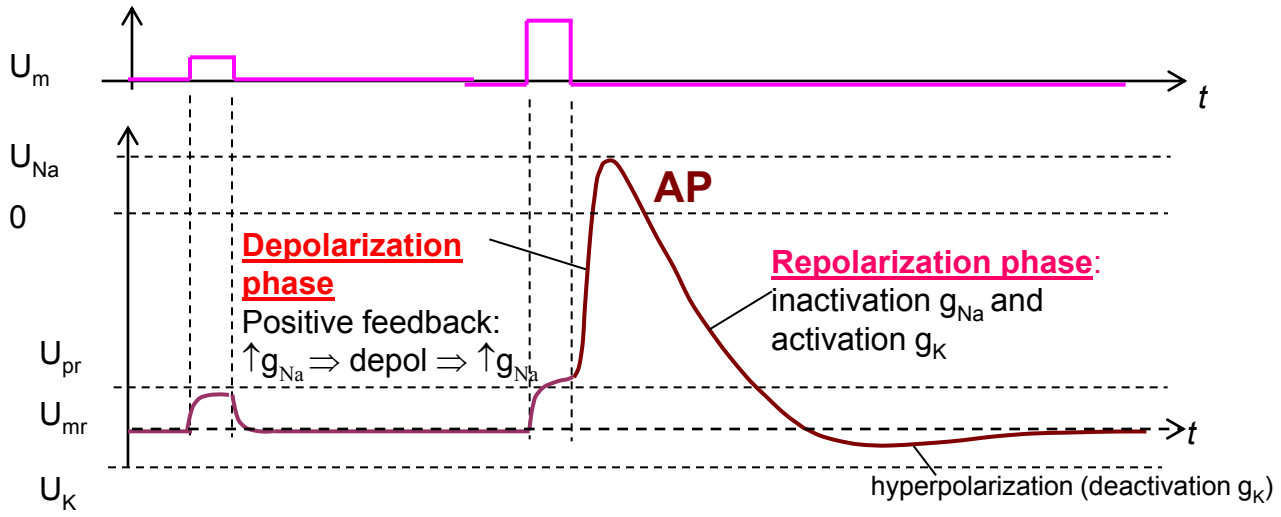
Action potential

- The concept of action potential denotes a fast change of the resting membrane potential caused by over-threshold stimulus which propagates into the adjacent areas of the membrane.
- This potential change is connected with abrupt changes in sodium and potassium ion channels permeability.
- The action potential can be evoked by electrical, chemical or mechanical stimuli which cause local decrease of the resting membrane potential.

Mechanism of action potential triggering



Mechanism of the action potential triggering in the cell membrane is an analogy of a monostable flip-flop electronic circuit ☺.

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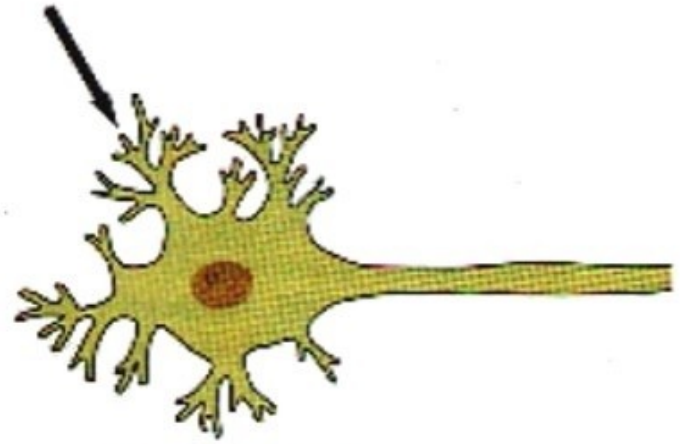
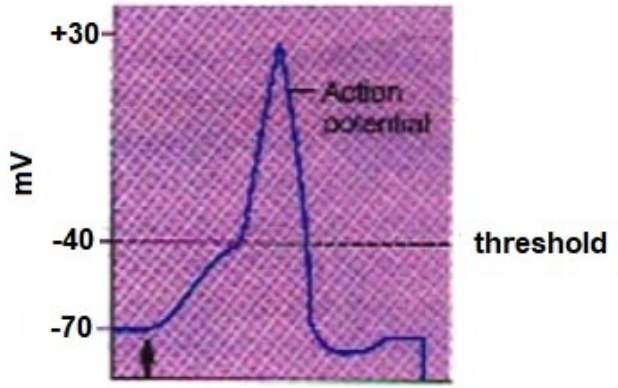
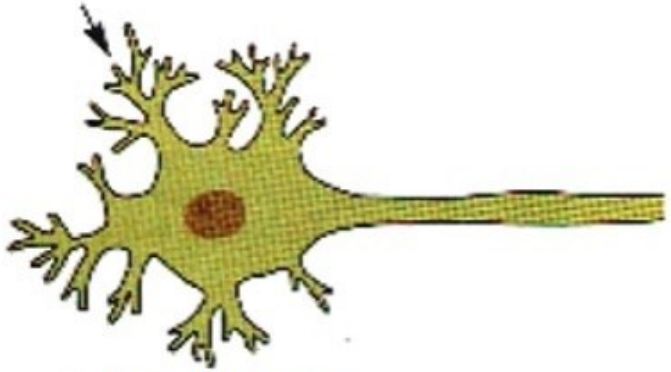
Origin of action potential

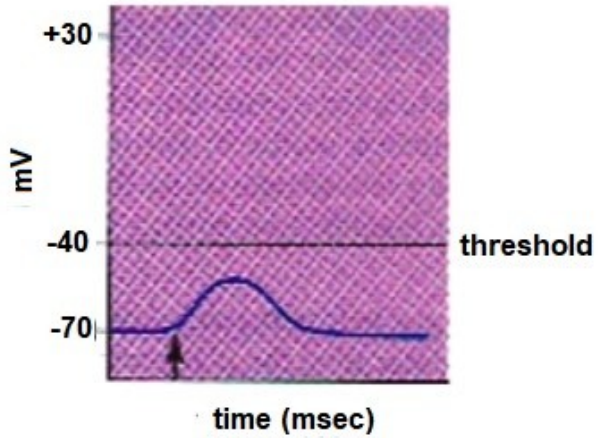


threshold stimulus





subthreshold stimlus



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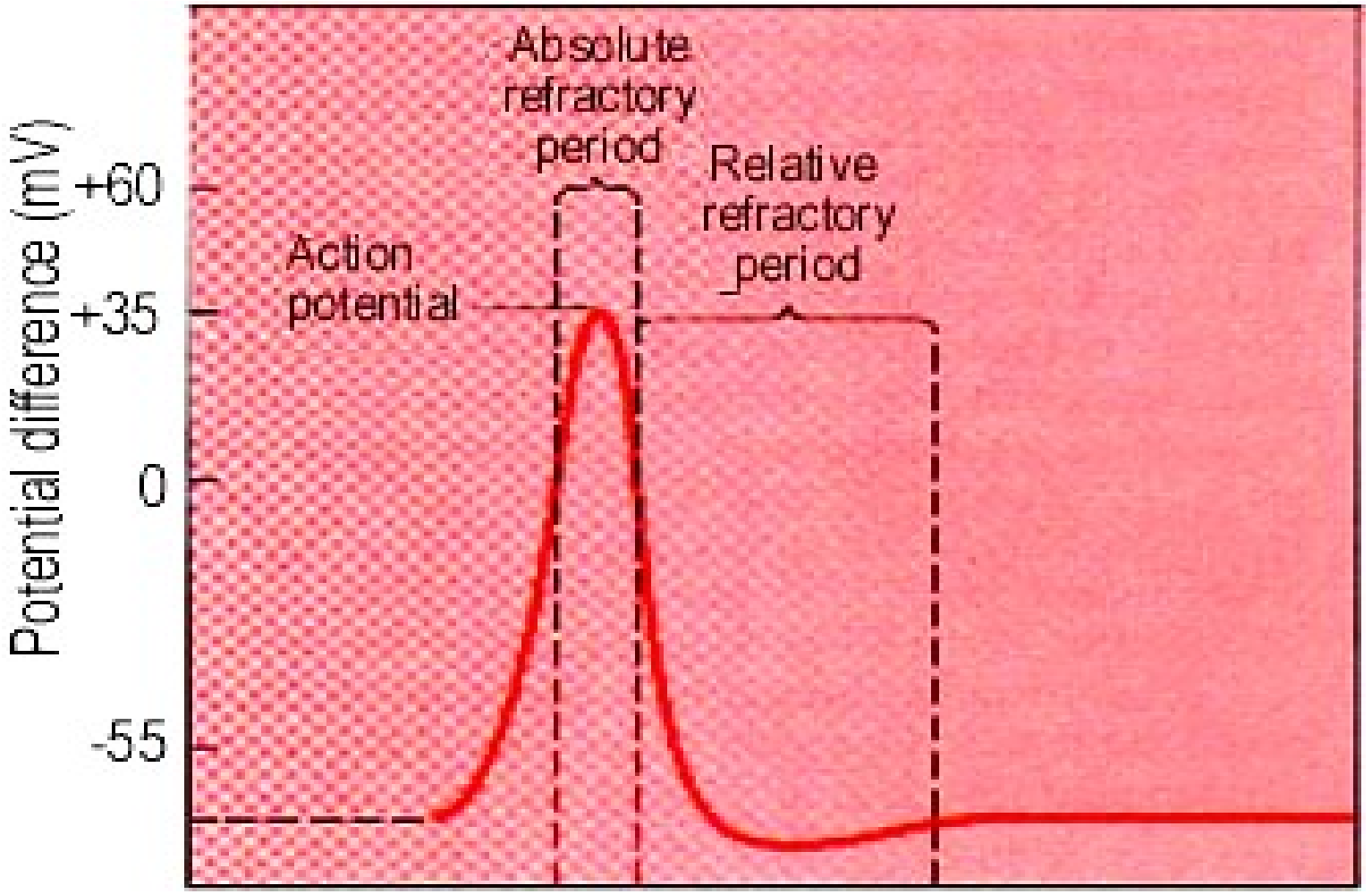
Refractory period



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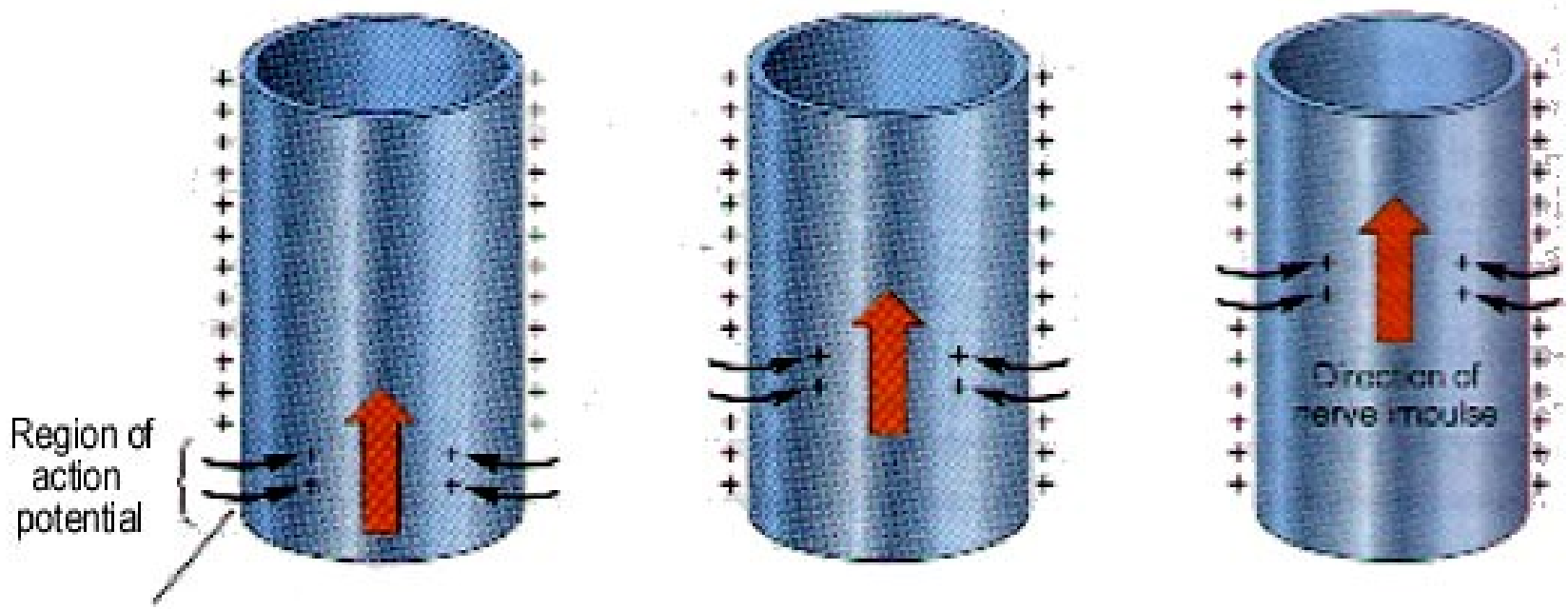
Action potential

- Changes in the distribution of ions caused by action potential are balanced with activity of ion pumps (active transport).
- The action potential belongs among phenomena denoted as „all or nothing“ response. Such response is always of the same size. Increasing intensity of the over-threshold stimulus thus manifests itself not as increased intensity of the action potential but as an increase in action potential **frequency (rate).**

Propagation of the action potential along the membrane



AP propagation is unidirectional because the opposite side of the membrane is in the refractory period.

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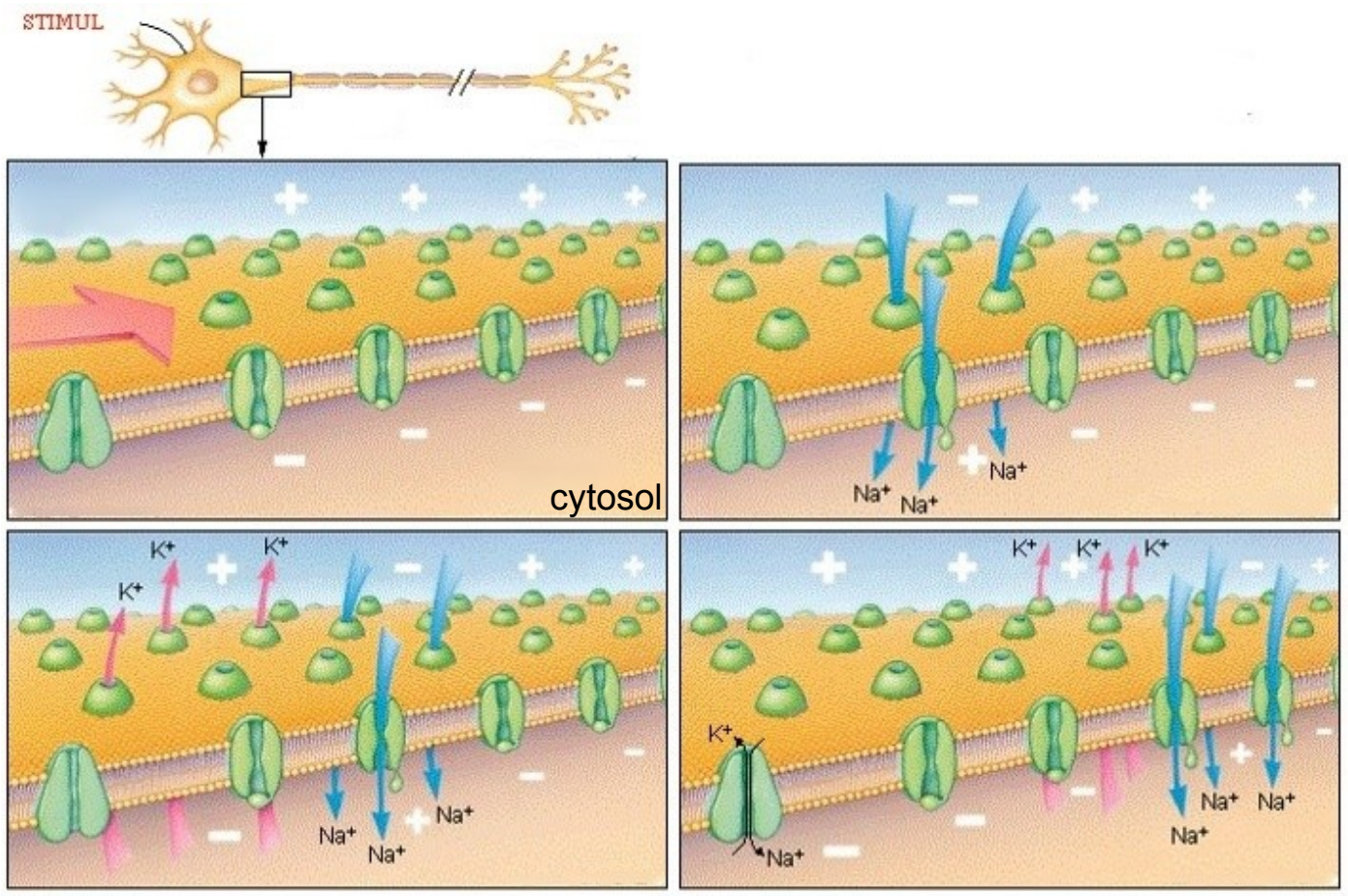
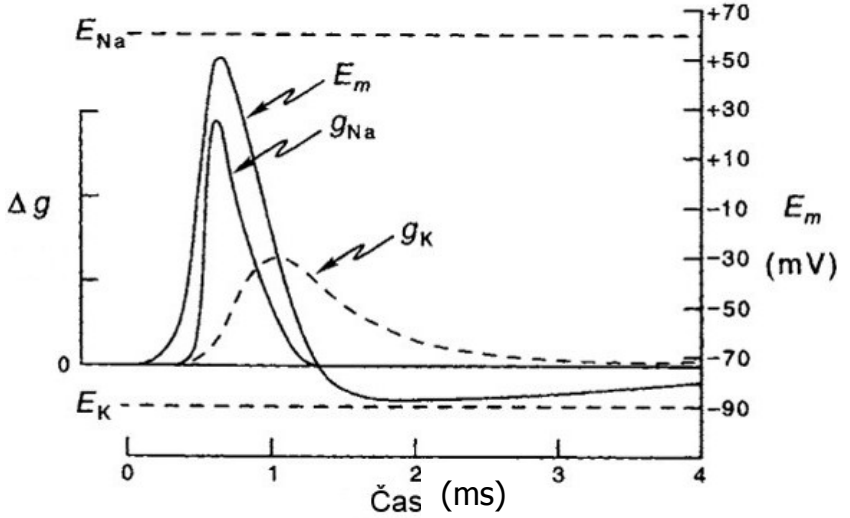
Propagation of the action potential along the neuron membrane (2)





E – means voltage here, potential
g = electric conductivity, it can be distinguished for individual ions

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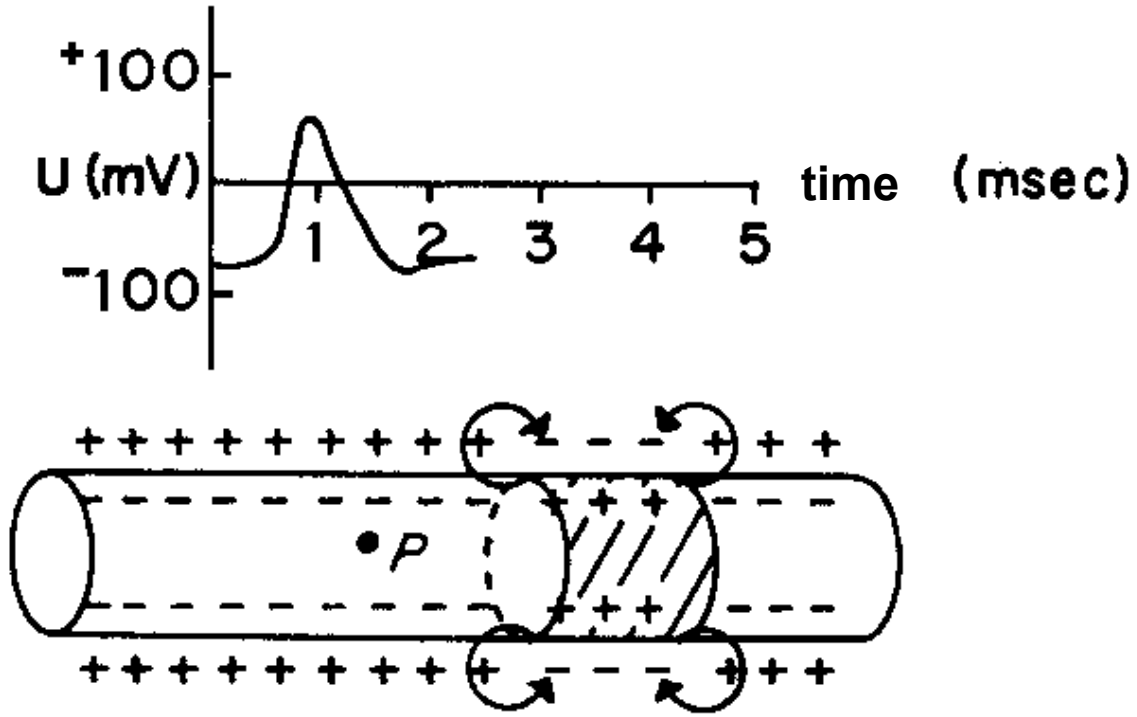
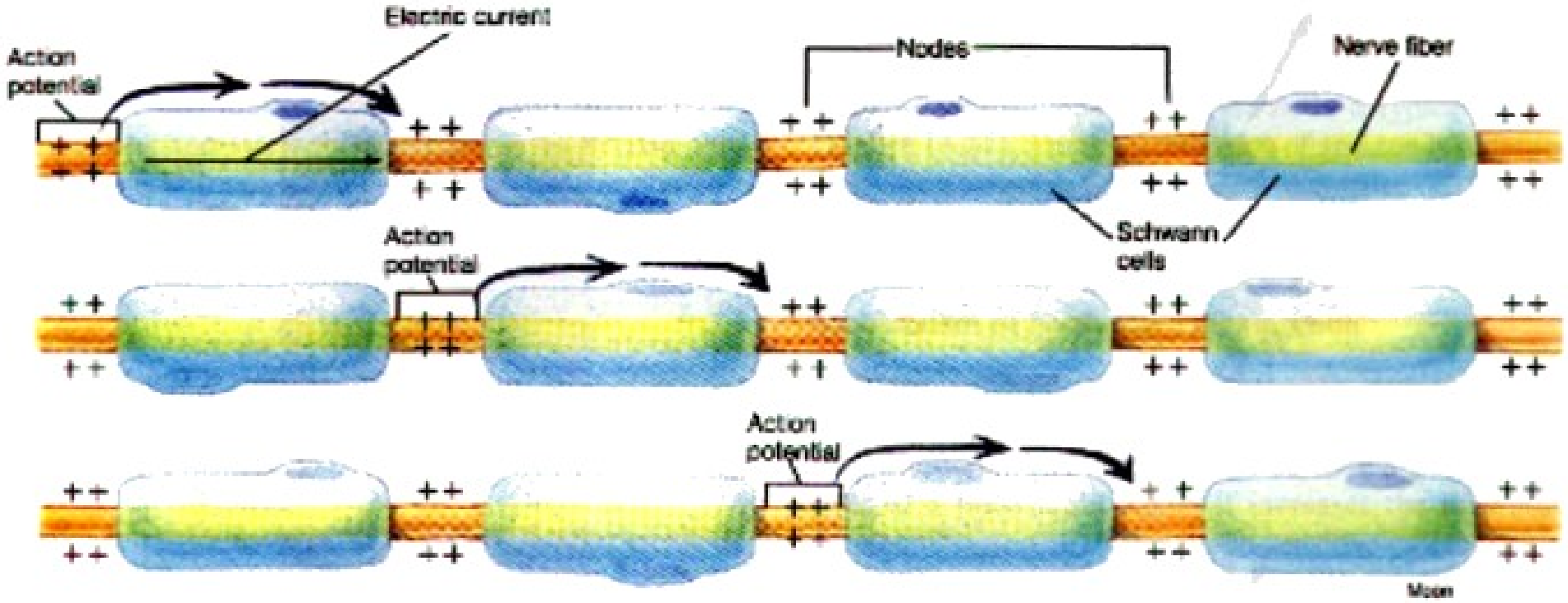
Propagation of AP and local currents



Conduction of action potential along the myelinated nerve fibre

Saltatory conduction



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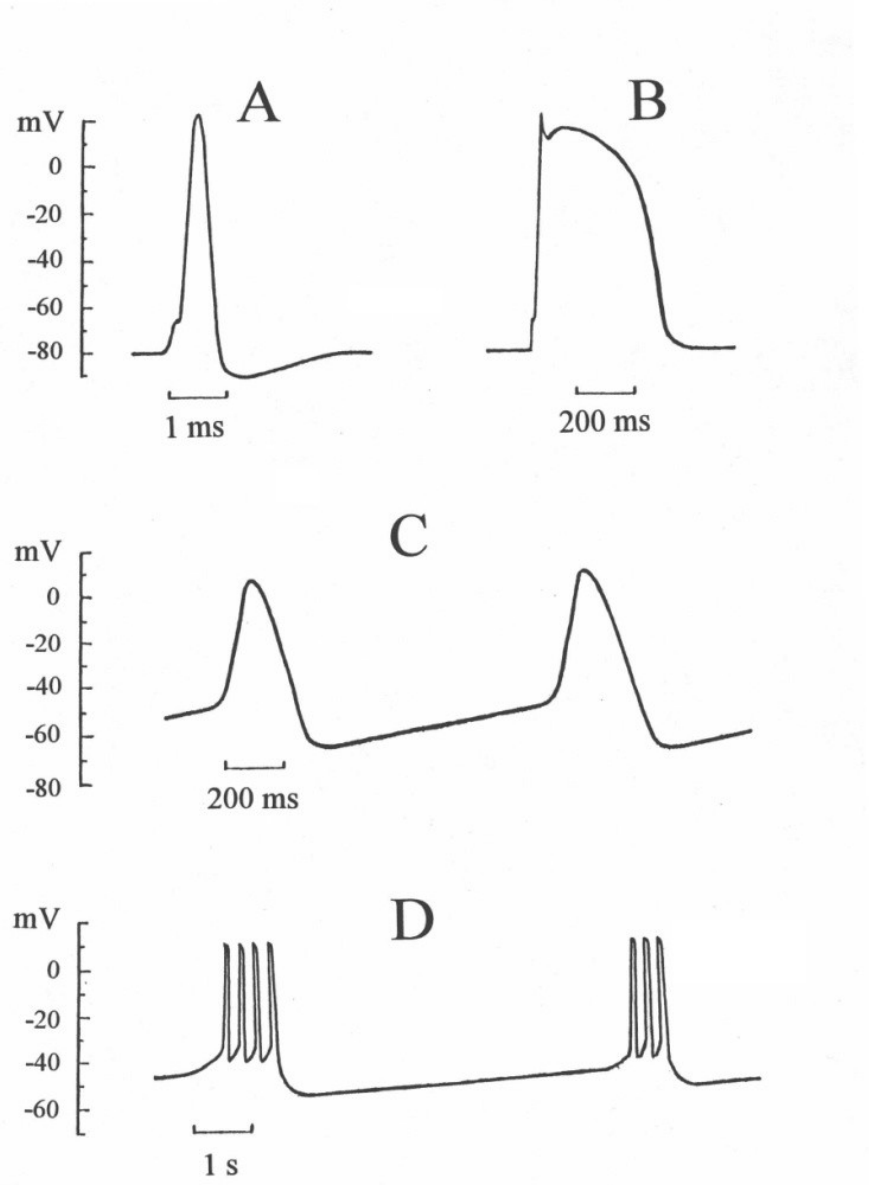
Examples of action potentials



A – nerve fibre
B – muscle cell of heart ventricle
C – cell of sinoatrial node
D – smooth muscle cell

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Synapse

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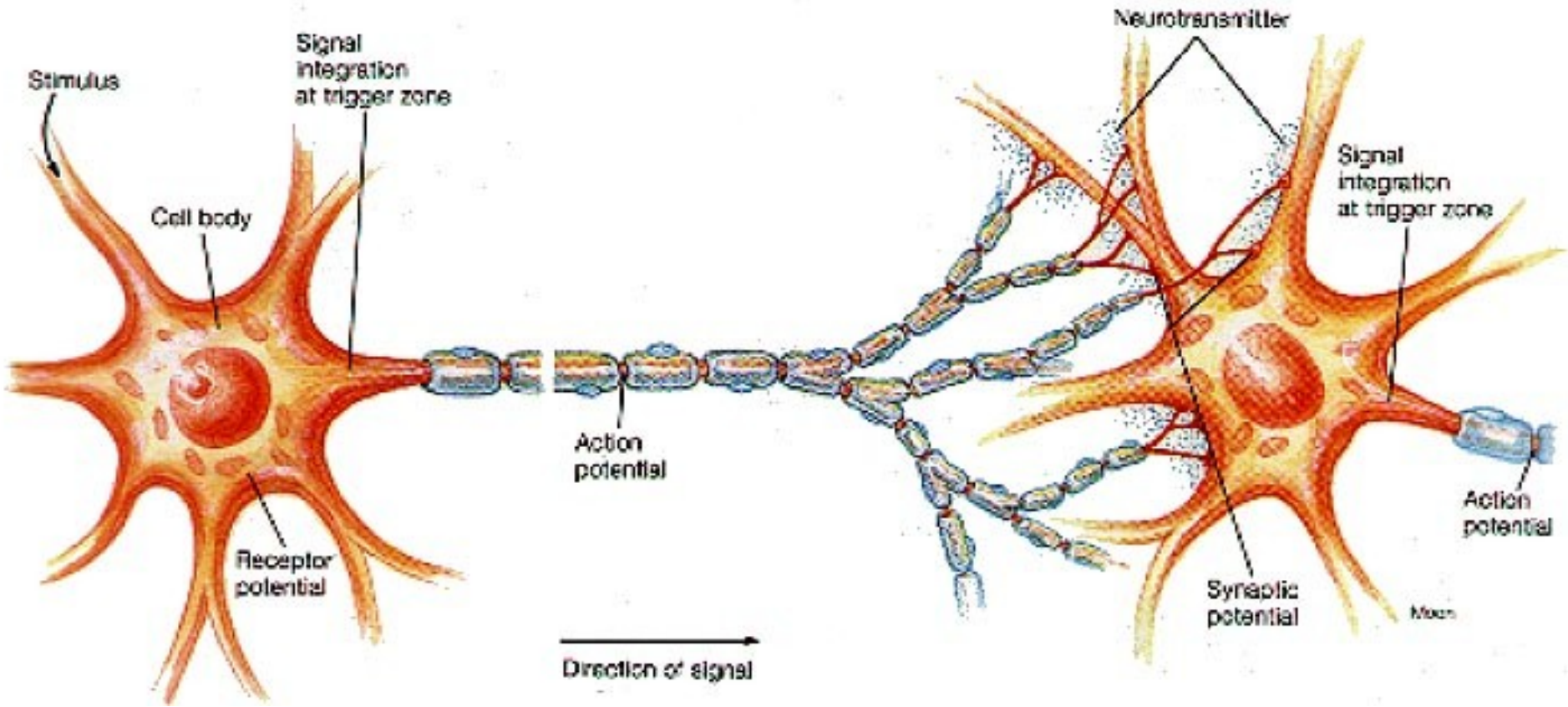
Definition

- **Synapse** is a specific connection between two neurons or between neurons an other target cells (e.g. muscle cells), which makes possible transfer of action potentials.

We distinguish:

- Electrical synapses (gap junctions) – close connections of two cells by means of ion channels. They enable a fast two-way transfer of action potentials.
- Chemical synapses – more frequent, specific structures, they enable one-way transfer of action potentials.

Transmission of action potentials between neurons



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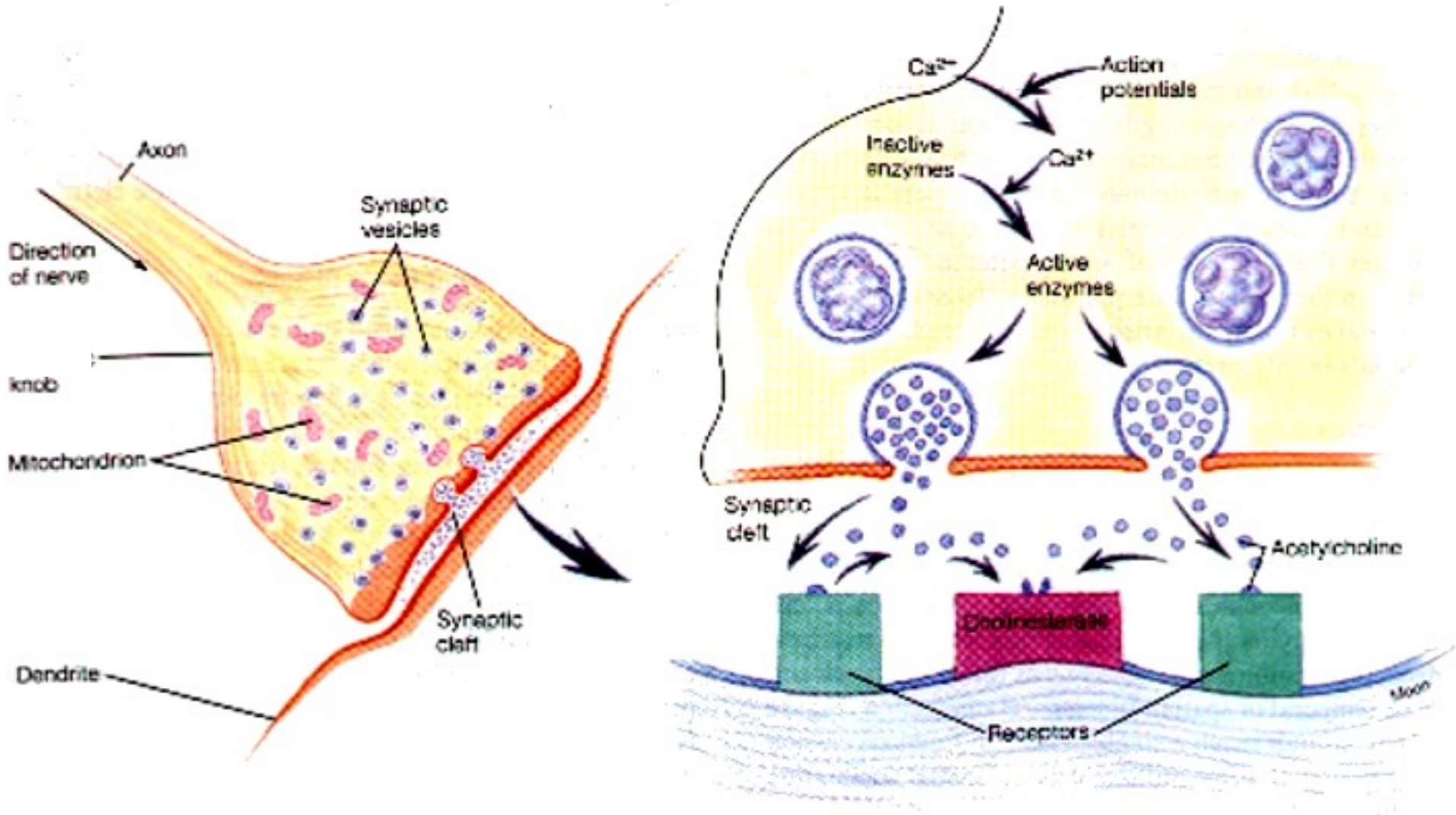
Chemical synapse



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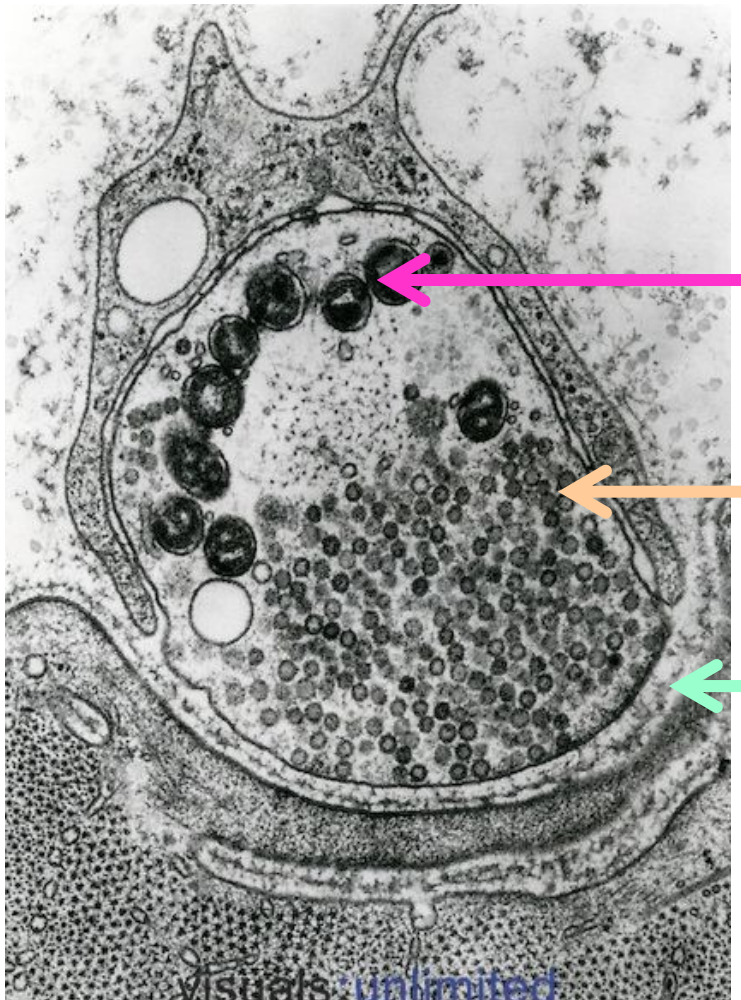
Chemical synapse – electron micrograph



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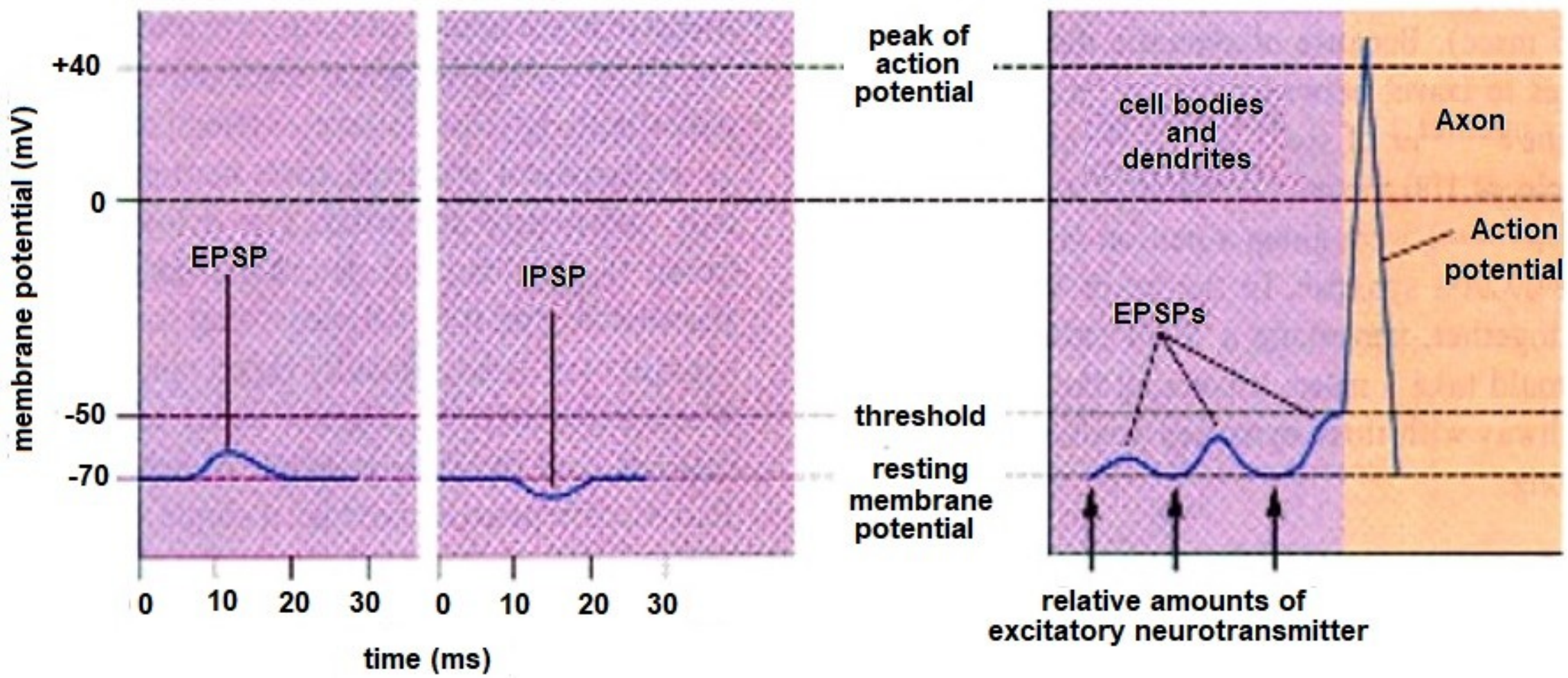
Synaptic mediators (neurotransmitters)

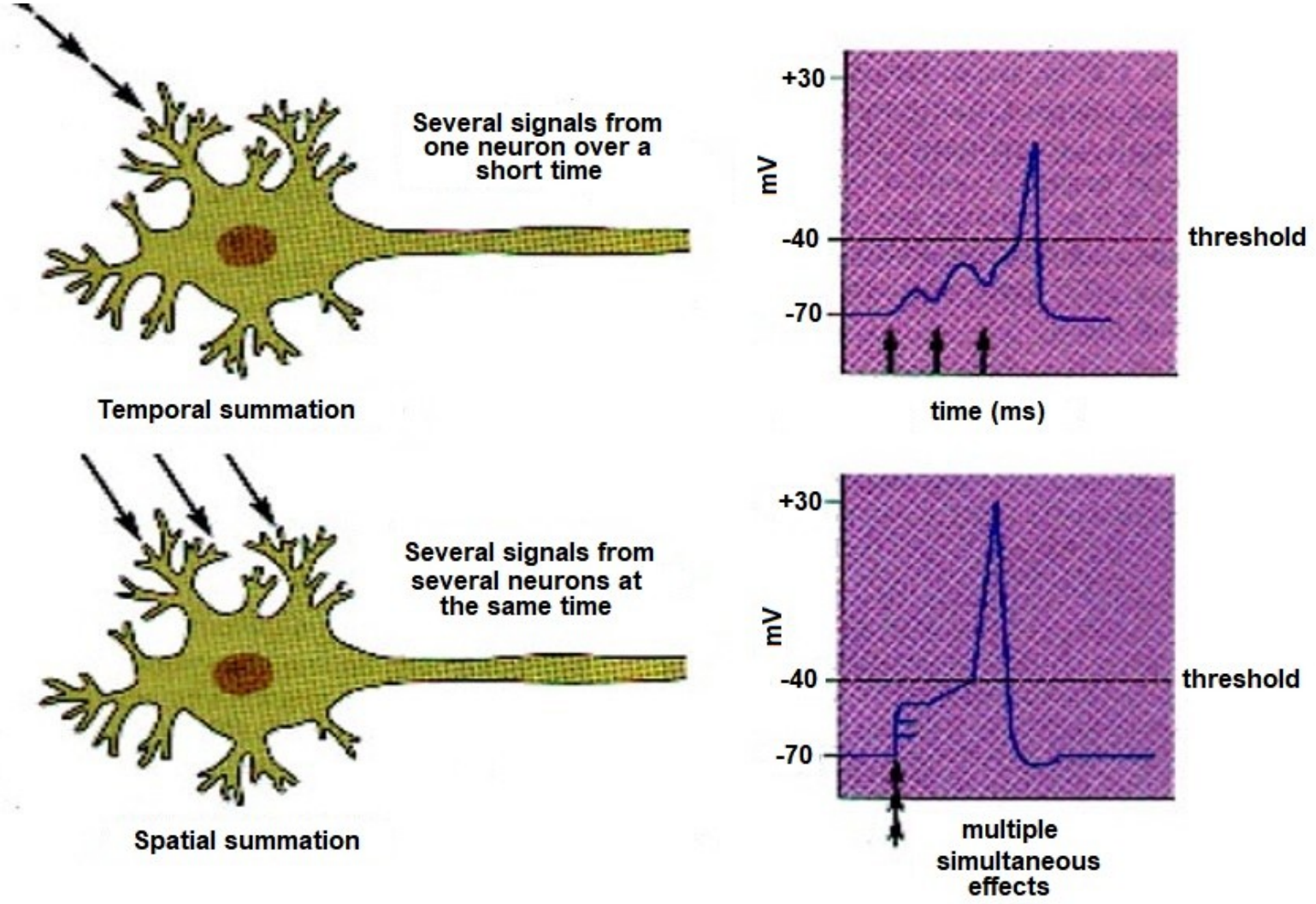
- The most frequent mediators (neurotransmitters) of excitation synapses are acetylcholine (in neuromuscular end plates and CNS) and glutamic acid (in CNS). Both compounds act as gating ligands mainly for sodium channels. Influx of sodium ions inside the cell evokes a membrane potential change in positive sense – towards a depolarisation of the membrane (excitation postsynaptic potential).

- Gamma-amino butyric acid (GABA) is a neurotransmitter of inhibitory synapses in brain. It acts as a gating ligand of chloride channels. Chloride ions enter the cell and evoke so a membrane potential change in negative sense – membrane hyperpolarization results (inhibitory postsynaptic potential).

Excitation and inhibition postsynaptic potential



Summation of postsynaptic potentials



Summary

- Electric phenomena on biological membranes play a key role in functioning of excitatory tissues (nerves, muscles)
- Resting membrane potential (correctly: membrane voltage) is a result of a non-equal distribution of ions on both sides of the membrane.
- It is maintained by two basic mechanisms: selective permeable ion channels and by transport systems – both these systems have protein character
- Changes of membrane voltage after excitation are denoted as action potentials
- Membrane undergoes two phases after excitation: depolarization – connected with influx of sodium ions into the cell - and subsequent repolarization – connected with efflux of potassium ions from the cell
- In the refractory period, the membrane is either fully or partly insensitive to stimulation
- Synapse is a connection of two cells which enables transmission of action potentials

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