Bioelectric phenomena.

- **Related practices: Amplifier, Pulse generator, ECG**
- **Related book chapters: III/4** ***Textbook: pp. 276-300.***

Balázs Kiss

kissb3@gmail.com

Nanobiotechnology and Single Molecule Research Group and

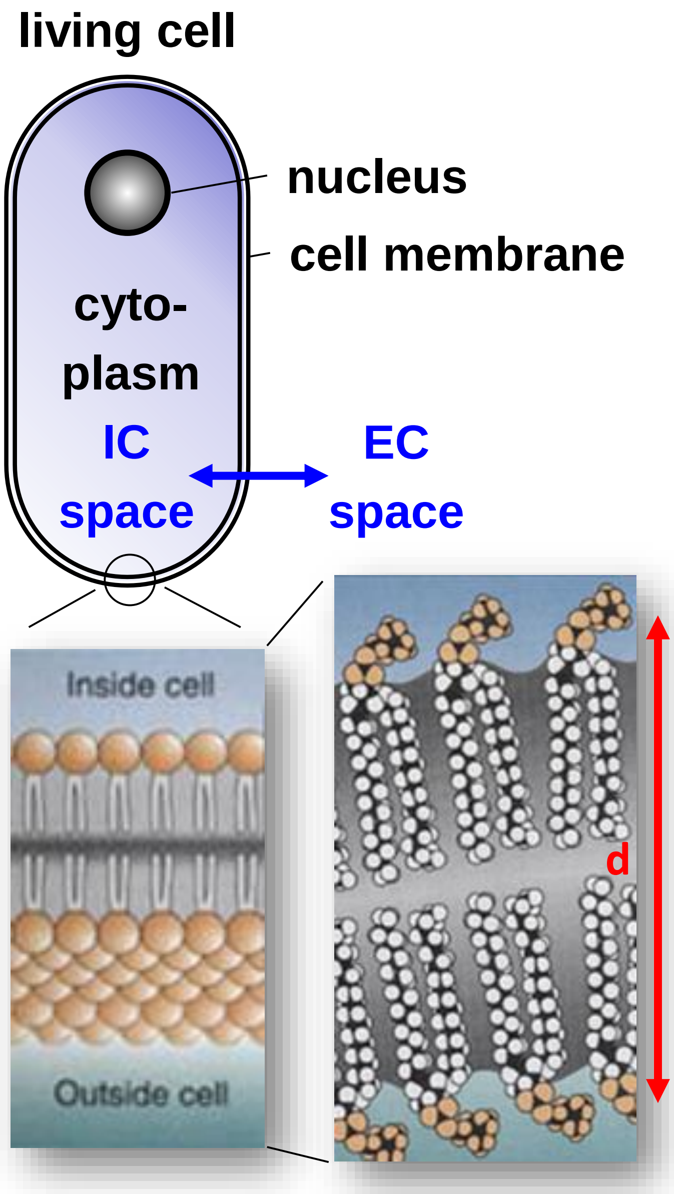
Muscle Mechanobiophysics Group

Department of Biophysics and Radiation Biology,

Semmelweis University

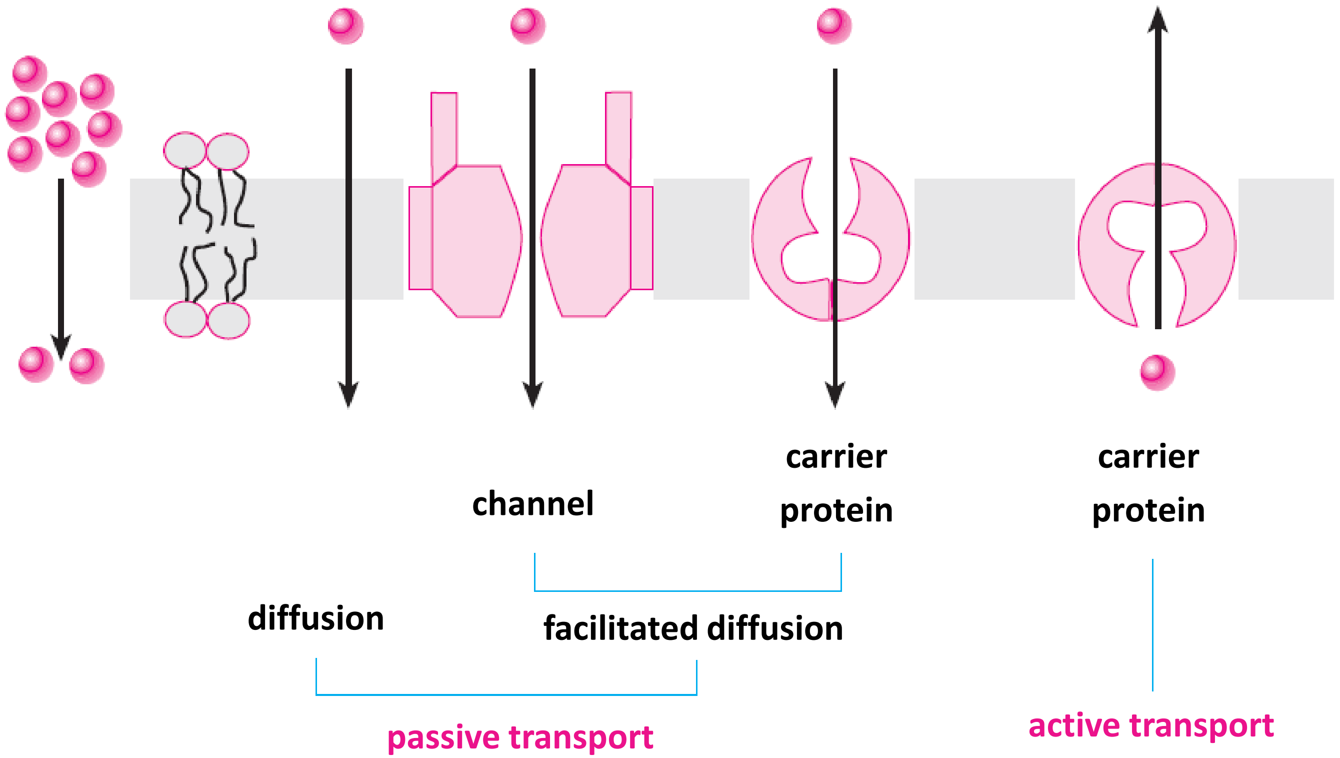
22. March 2023.

Physical properties of the cell membrane



- **Noncovalent, cooperative structure:** phospholipid bilayer, vesicle formation, additional components (e.g. cholesterol, proteins)
- **Thin, layered**: d ~ 5 nm
- **Asymmetric:** the two sides of the membrane have different properties
- **Permeability:** impermeable to ions, permeable to water
- **Fluidity:** melting temperature (T_m)
- **Lateral diffusion:** lateral movement of lipid-, and protein molecules
- **Flip-flop:** phospholipid translocation between the two layers (low probability)
- **Flexibility, elasticity:** see distortion / deformation of erythrocytes in the capillary

Transport across biological membranes



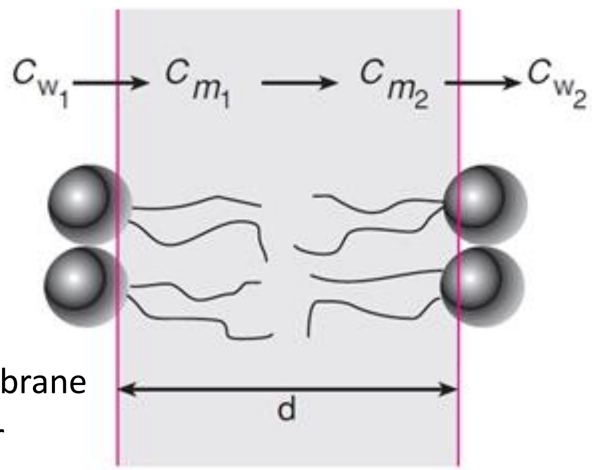
- **Passive diffusion:** „real", classical diffusion (Fick's first law)
- **Facilitated or mediated diffusion:** through biological membranes, through/with protein(like) mediator molecules (carrier or channel proteins)
- **Active transport:** the particle is transported against a gradient (chemical/electrochem.)

Passive diffusion across the membrane

IC space (aqueous phase)

membrane

EC space (aqueous phase)



„m”: membrane

„w”: water

Fick's first law:

$$J_m = -D \cdot \frac{\Delta c}{\Delta x} = -D_m \frac{c_{m2} - c_{m1}}{d}$$

D_m: diffusion coefficient within the membrane

Permeability constant: p_m, [m/s]

$$p_m = \frac{D_m}{d}$$

the thickness of the membrane is usually not precisely known

Partition coefficient: K

(between the membrane and aqueous phases)

$$\frac{c_{m1}}{c_{w1}} = \frac{c_{m2}}{c_{w2}} = \text{const.} = K$$

in the case of stationary diffusion

$$J_m = -p_m \cdot K(c_{w2} - c_{w1}) = -p(c_{w2} - c_{w1})$$

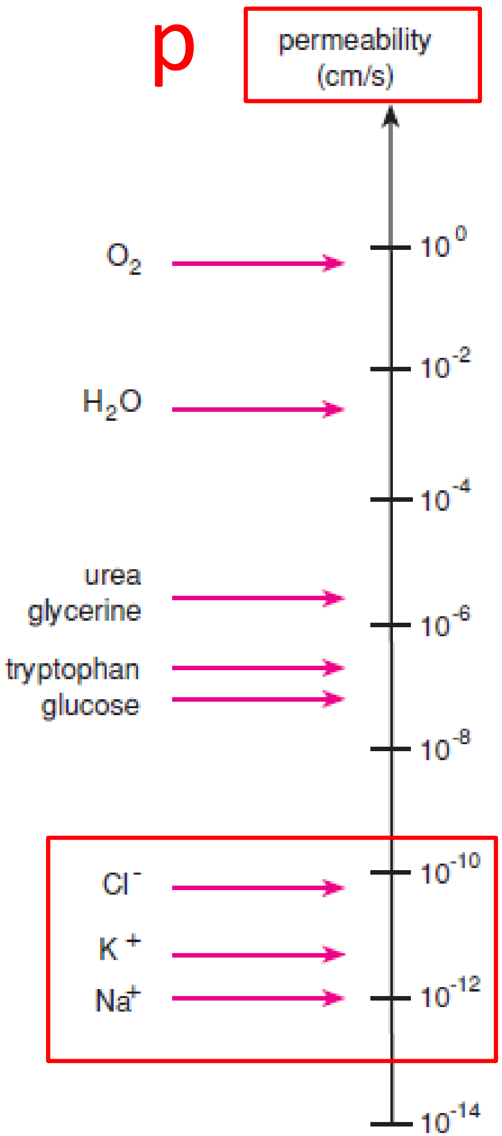
Aggregated permeability constant: p, [m/s]

$$p = K \cdot p_m$$

„permeability”

considers the diffusion coefficient, membrane thickness and concentrations inside and outside of the membrane

Passive diffusion of particles



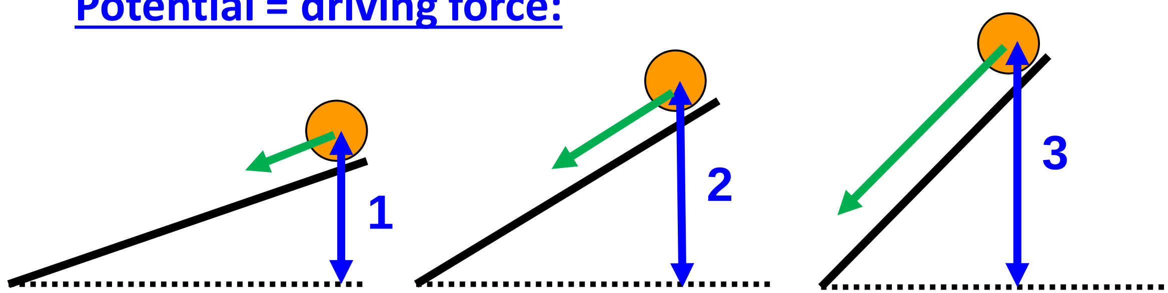
Onsanger equation:

$$J = L \cdot X$$

matter flow density	conductivity coefficient	gradient of an intensive quantity

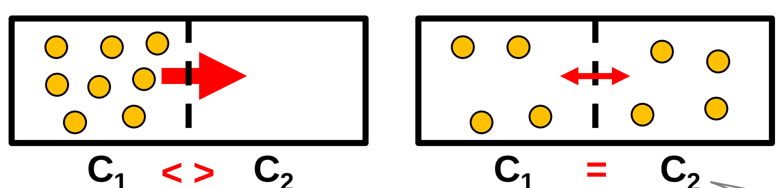
The driving force of transport is the chemical potential gradient.

Potential = driving force:



also known as molar free enthalpy

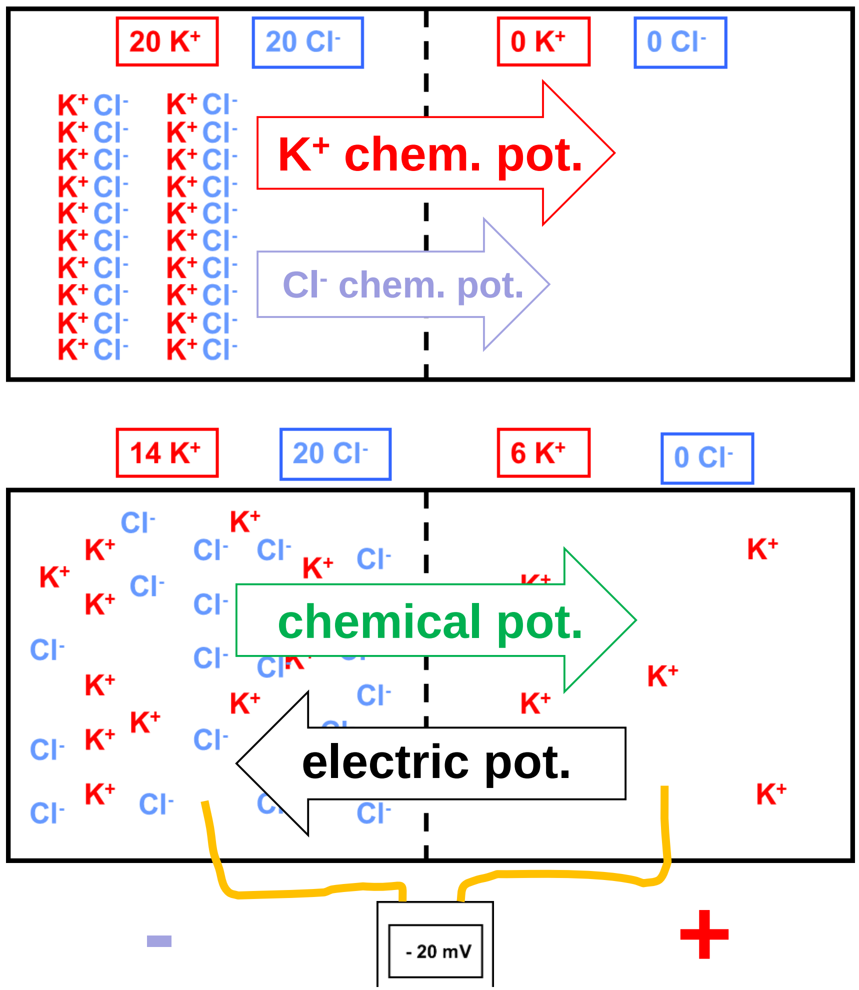
Chemical potential: μ $\mu = \mu_0 + RT \cdot \ln(c)$



μ_0: standard chemical potential

in thermal equilibrium

Passive diffusion of ions: electrochemical potential



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

Assume that the membrane is only permeable to K^+ ($p_{Cl^-}=0$).

In equilibrium:

- concentration difference
- electric potential difference

exist between the two compartments.

- **the chemical and electric potentials are of the same magnitude but oppositely directed.**

Electrochemical potential: μ_e, [J/mol]

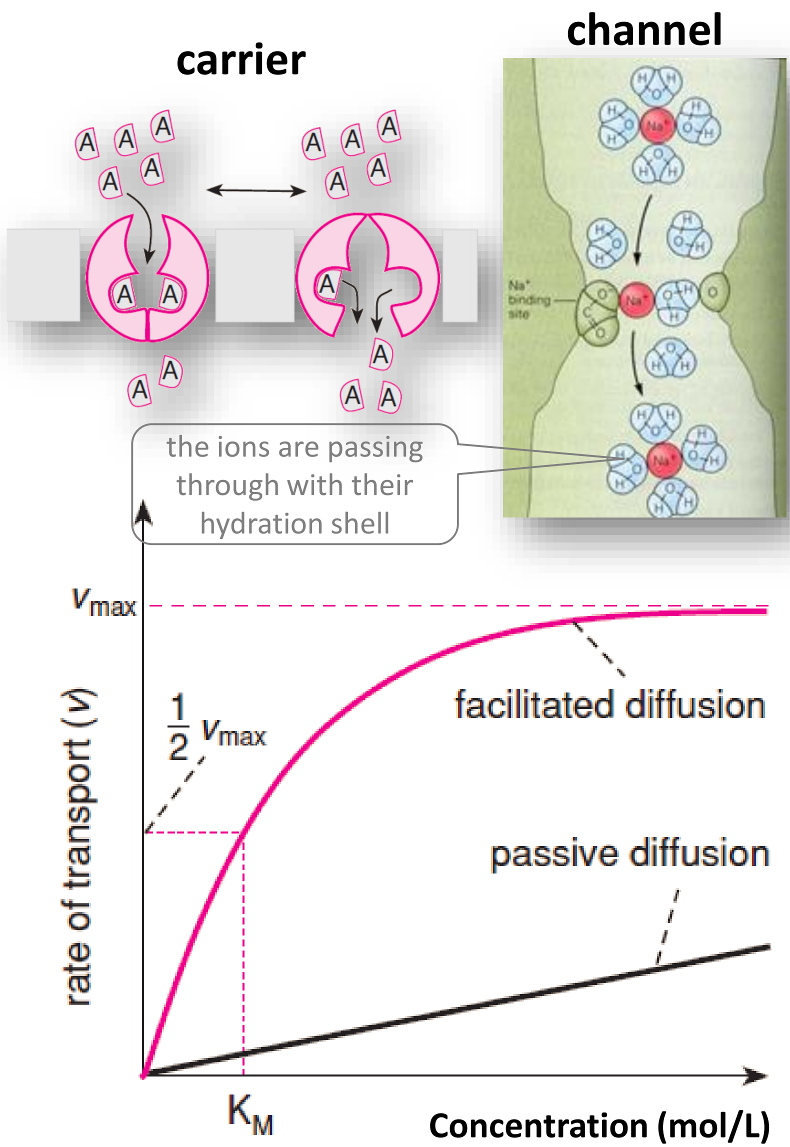
$$\mu_e = \underbrace{\mu}_{\text{chemical}} + \underbrace{zF\varphi}_{\text{electric}}$$

z: charge of the ion

F: Faraday constant

φ: electric potential

Properties of the facilitated diffusion

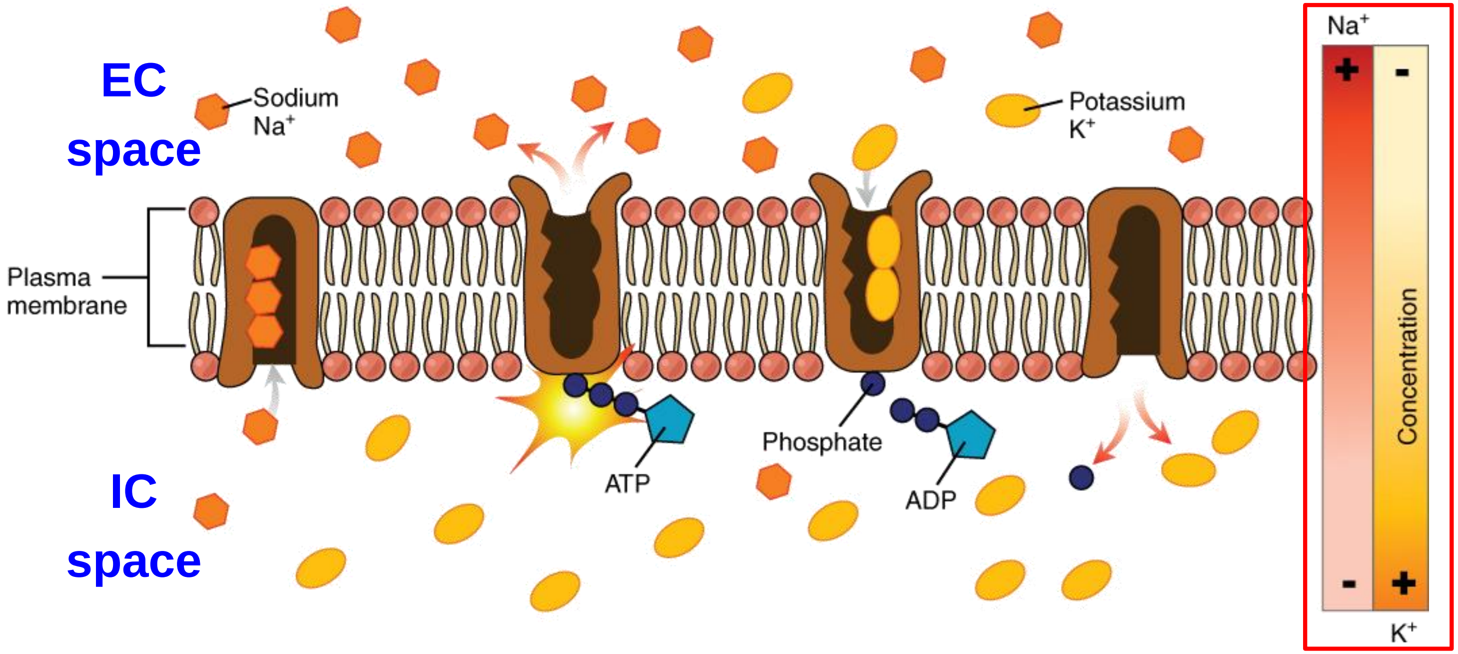


- **Faster, than the passive diffusion** (what is expected based on Fick's first law)
- **Selective**: works only for a given particle or for molecules sharing structural similarity
- **Can be saturated:** is realized through a limited number of mediator molecules (carrier or channel)
- **Can theoretically work in both directions:** the direction is determined by the sign or direction of the (electro)chemical potential gradient of the transported molecule
- **Can be selectively inhibited:** with inhibitors targeting the mediator molecules
- Ionophores: mobile ioncarriers or channel-forming molecules. Application: antibiotics

Active transport

- **Transpost of molecules against their (electro)chemical gradient, energetically:**
  - **ATP-driven**: ATP-ases, they hydrolyze ATP
  - **light-gated** (e.g. channelrhodopsin-2: non-selective cation channel)
  - **coupled transporter**: couples the transport of a substance with sufficient electrochemical gradient to the transport of another molecule against its gradient

- **According to the numbers of the transported molecules:**
  - **uniporter**: translocates only one molecule across the membrane
  - **symporter**: transport the particles in the same direction
  - **antiporter**: transport the particles in the opposite direction, e.g. **Na^{+}-K^{+} ATP-ase**:



ATP!

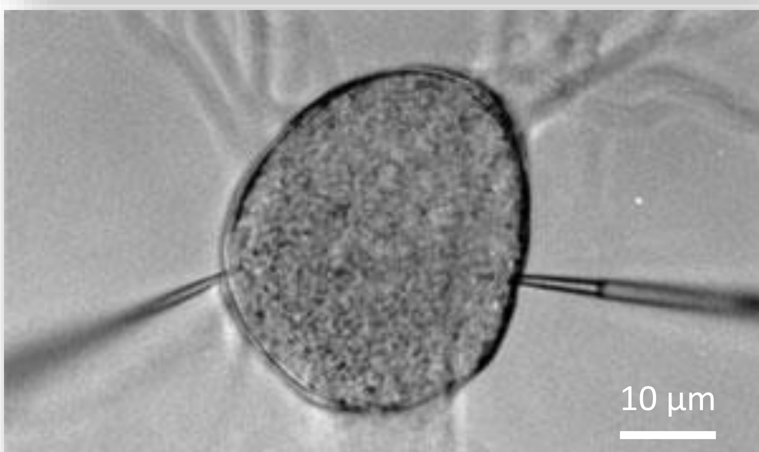
3 Na^{+} out

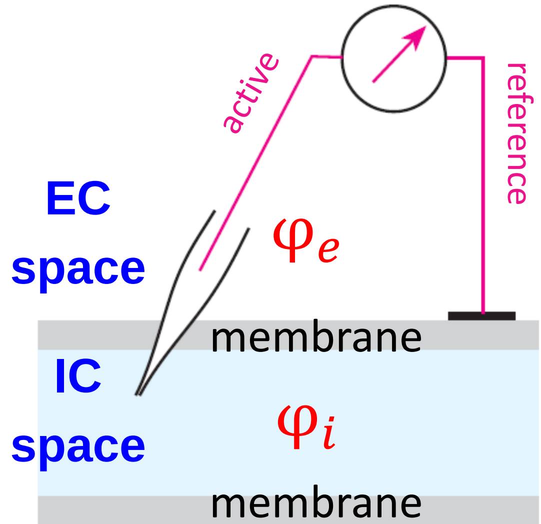
2 K^{+} in

Resting membrane potential

<u>Measurement:</u> with microelectrode pairs

- active
- reference





voltage = potential difference = „potential"

<u>Observation:</u> $\Delta\varphi = \varphi_i - \varphi_e < 0$

Cell	Δφ (mV)
squid giant axon	-62
frog muscle	-92
rat muscle	-92

The intracellular space is more negative.

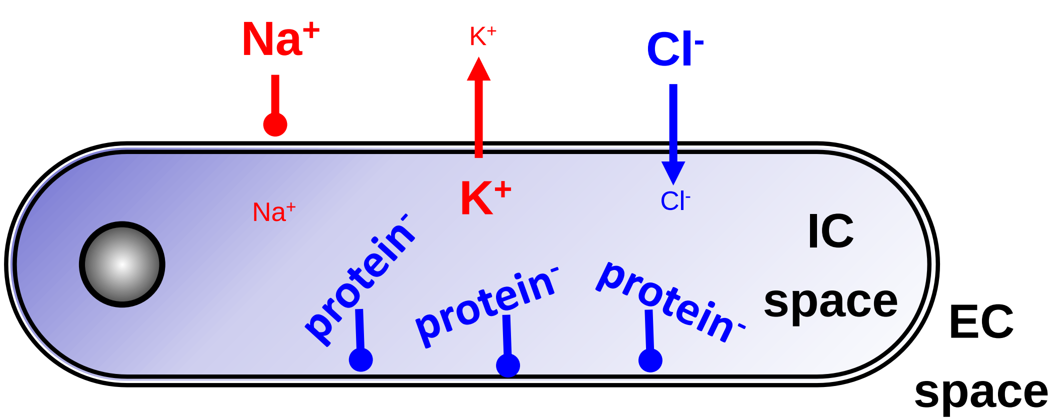
Resting membrane potential

Further observation: different ion concentrations on both sides of the membrane

	intracellular concentration (mmol/l)			extracellular concentration (mmol/l)		
cell	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-
squid giant 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

Considering the ion distribution shown in the table above which physical model gives the best approximation of the resting membrane potential?

Model #1: Donnan-model: equilibrium ion distribution, additional protein anions (IC)



- The membrane is impermeable to certain ions ($p_{protein^-} = 0$).
- Electrochemical equilibrium is assumed.

Resting membrane potential

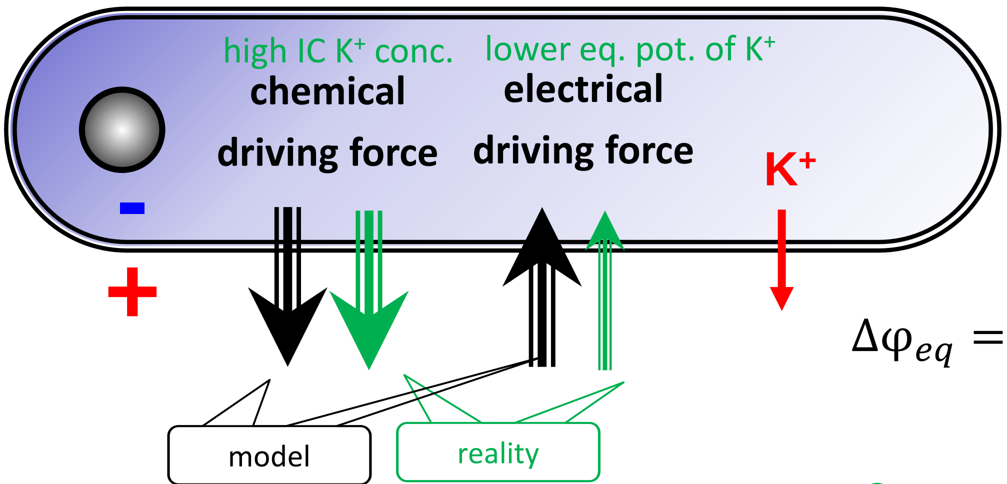
This is done by applying the Nernst-equation...

Equilibrium potential: calculated based on the #1 Donnan-model...

Nernst equation: $\Delta\varphi = \varphi_2 - \varphi_1 = -\frac{RT}{F} \ln \frac{c_2}{c_1}$

let's calculate it for the K^+ ions...

	intracellular concentration (mmol/l)			extracellular concentration (mmol/l)		
cell	Na+	K+	Cl-	Na+	K+	Cl-
squid giant axon	72	345	61	455	10	540



the Nernst-eqation for the K^+ ions

$$\Delta\varphi_{eq} = -\frac{RT}{F} ln \frac{c_i}{c_e}$$

$$\Delta\varphi_{eq} = -\frac{8{,}31 * 293}{96500} ln \frac{345}{10} = \text{-0,089 V} = \boxed{\text{-89 mV}}$$

Measured membrane potential: -62 mV

Outward flux of K^+ should happen at -62 mV

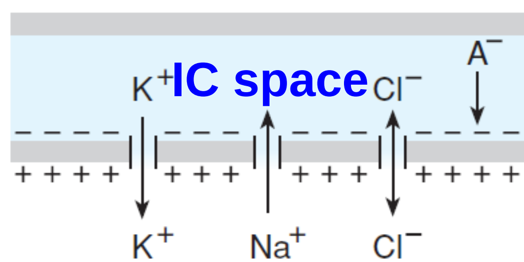
The equlibrium model does not correctly describe the real situation!

The Goldman-Hodgkin-Katz (GHK) equation

Cell	$\Delta\varphi_{equilibrium}$ (mV) using the Nernst equation			$\Delta\varphi_{membrane}$ (mV)
	Na^+	K^+	Cl^-	
squid giant axon	+46	-89	-55	-62
frog muscle	+45	-101	-87	-92
rat muslce	+64	-93	-85	-92

No equilibrium at rest but the transport processes contiune:

- outward flux of K^+
- inward flux of Na^+
- minor outward flux of Cl^-



- **active transport: requires energy (ATP)**

Transport model #2: continuous diffusion of different ions with different permeability (p)

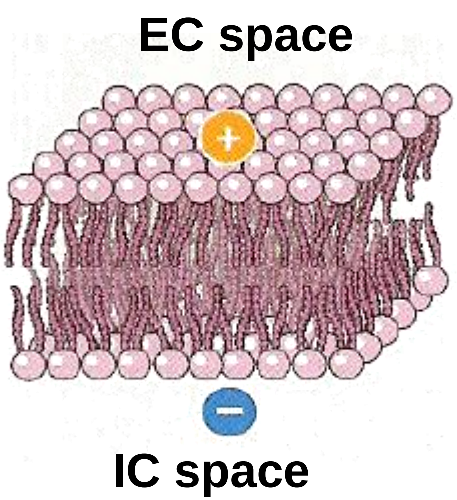
$$\Delta\varphi = \varphi_i - \varphi_e = -\frac{RT}{F} ln \frac{p_{Na}c_{Na}^i + p_K c_K^i + p_{Cl}c_{Cl}^e}{p_{Na}c_{Na}^e + p_K c_K^e + p_{Cl}c_{Cl}^i} = \text{-91 mV}$$

in frog muslce

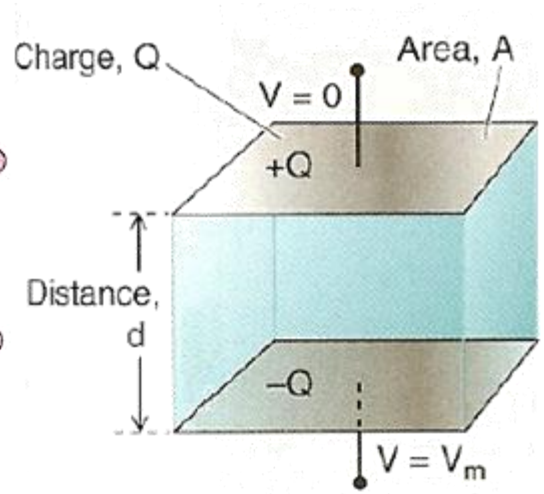
The calculation using the GHK equation is in agreement with the measurements.

The electric model of the cell membrane

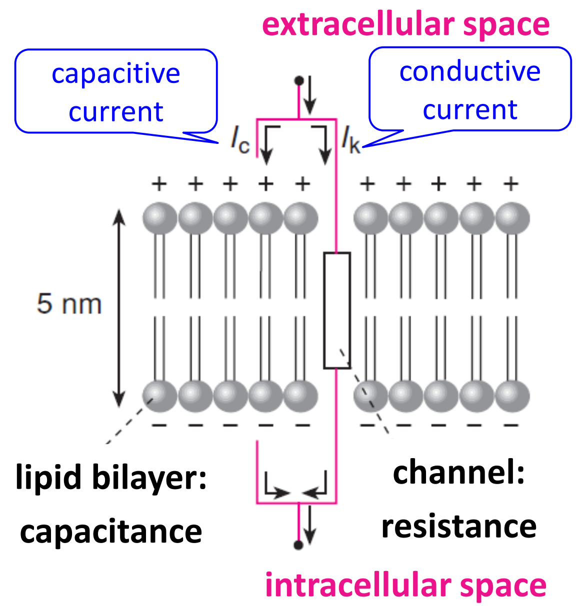
cell membrane



capacitor



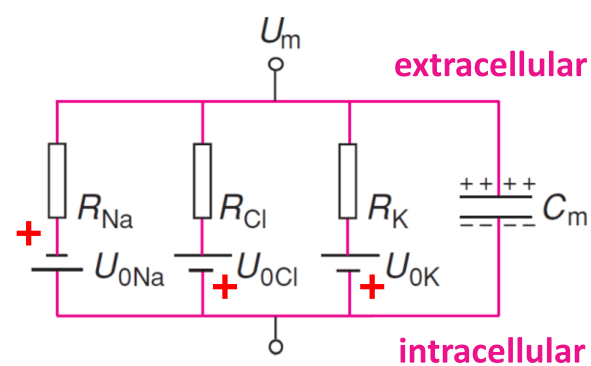
electric model



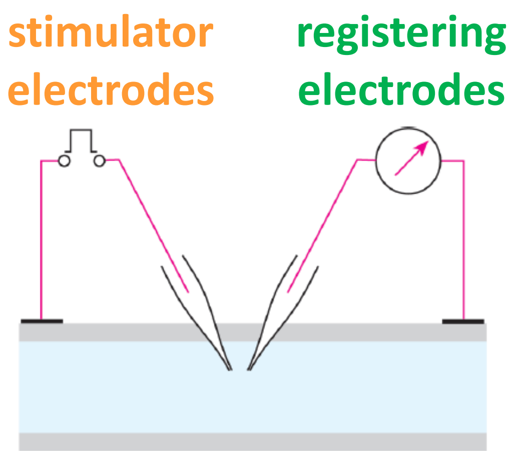
- different **transmembrane resistance (R)** in the case of the different ion channels
- **electric conductivity**: G (unit: Siemens) $G = \frac{1}{R}$

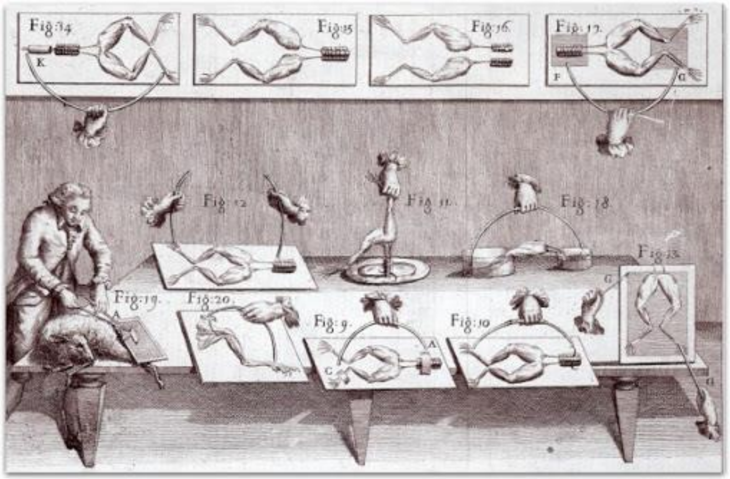
  proportional to **permeability (p)**

- **specific conductivity**: sigma (unit: 1/(Ω·m²)) $\sigma = \frac{1}{R \cdot A}$



The change of the resting potential in time





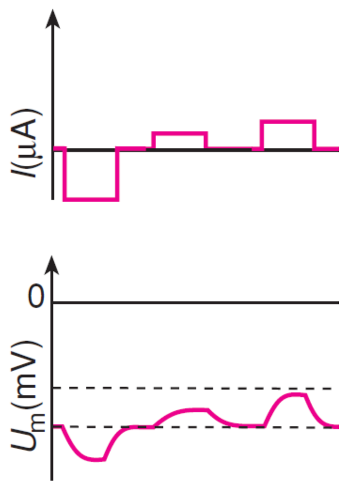
Local (electrotonic) changes of the membrane potential:

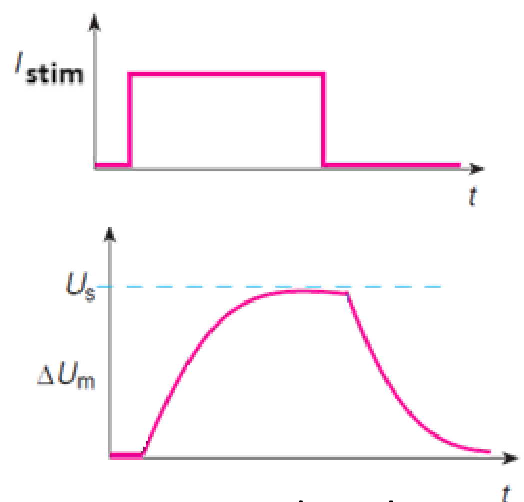
Stimulation

I_{stim}

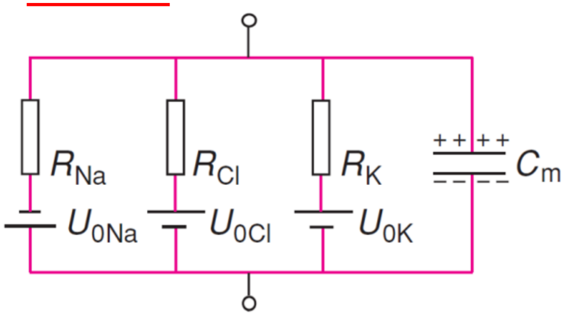
Response

$\Delta\varphi_m$





Model: RC-circuit

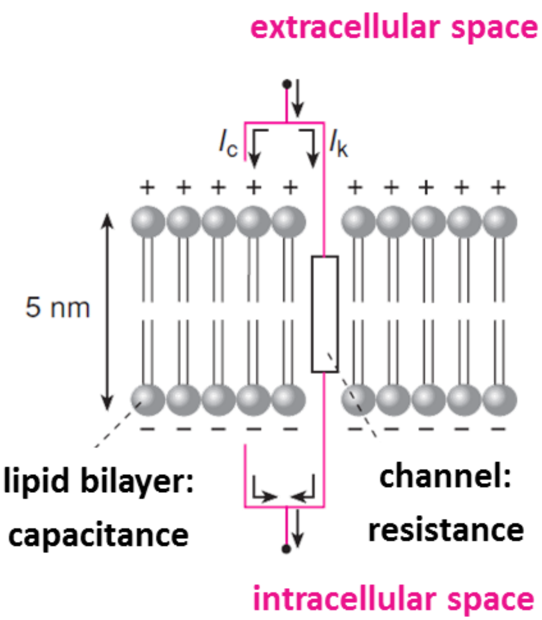


Time-constant: tau: τ [s]

$\tau = R_m \cdot C_m$

The amplitude of the response is proportional to the stimulating current, but shows a characteristic delay.

Electric properties of the membrane



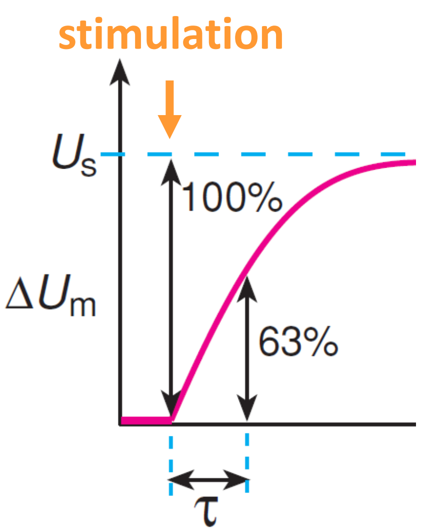
Currents across the membrane:

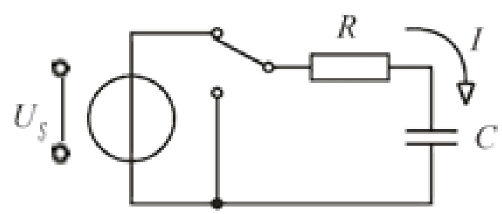
- conductive: I_k
- capacitive: I_C
- stimulating: I_{stim} negative with the influx of + charges

Based on the transport model for the resting state:

$$I_C + I_k - I_{stim} = 0$$

RC: „charge"

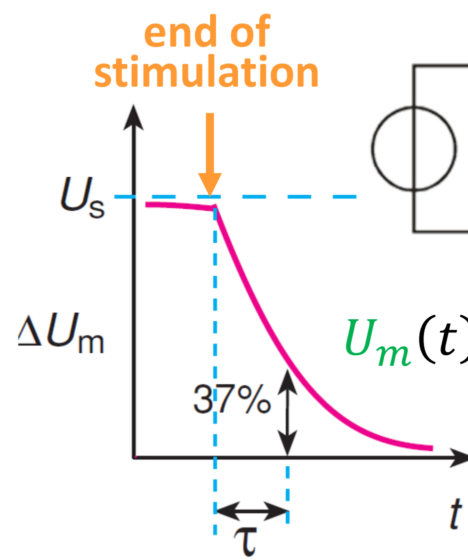


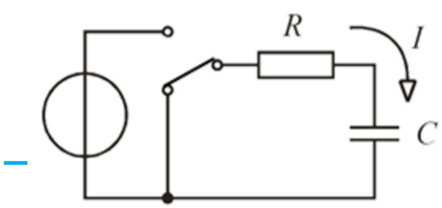


$$U_m(t) = U_S \cdot \left(1 - e^{-\frac{t}{\tau}}\right)$$

saturation voltage

RC: „discharge"



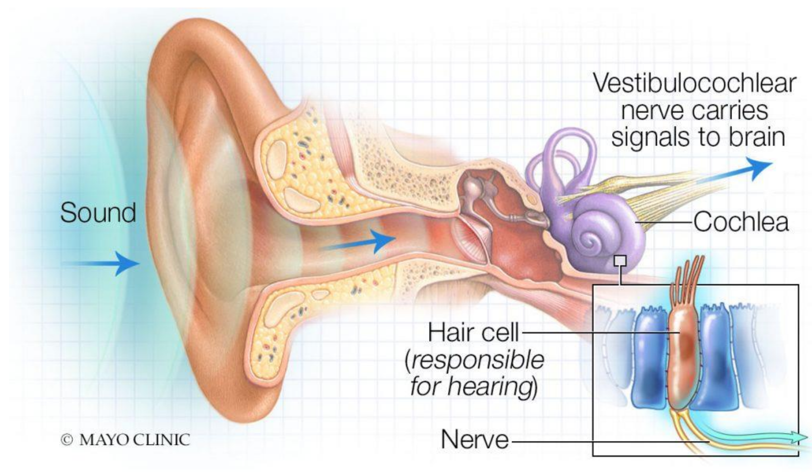


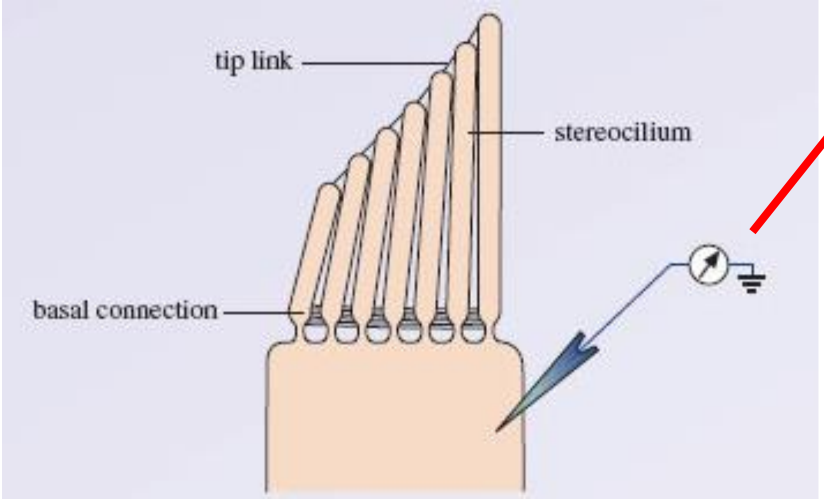
$$U_m(t) = U_S \cdot e^{-\frac{t}{\tau}}$$

saturation voltage

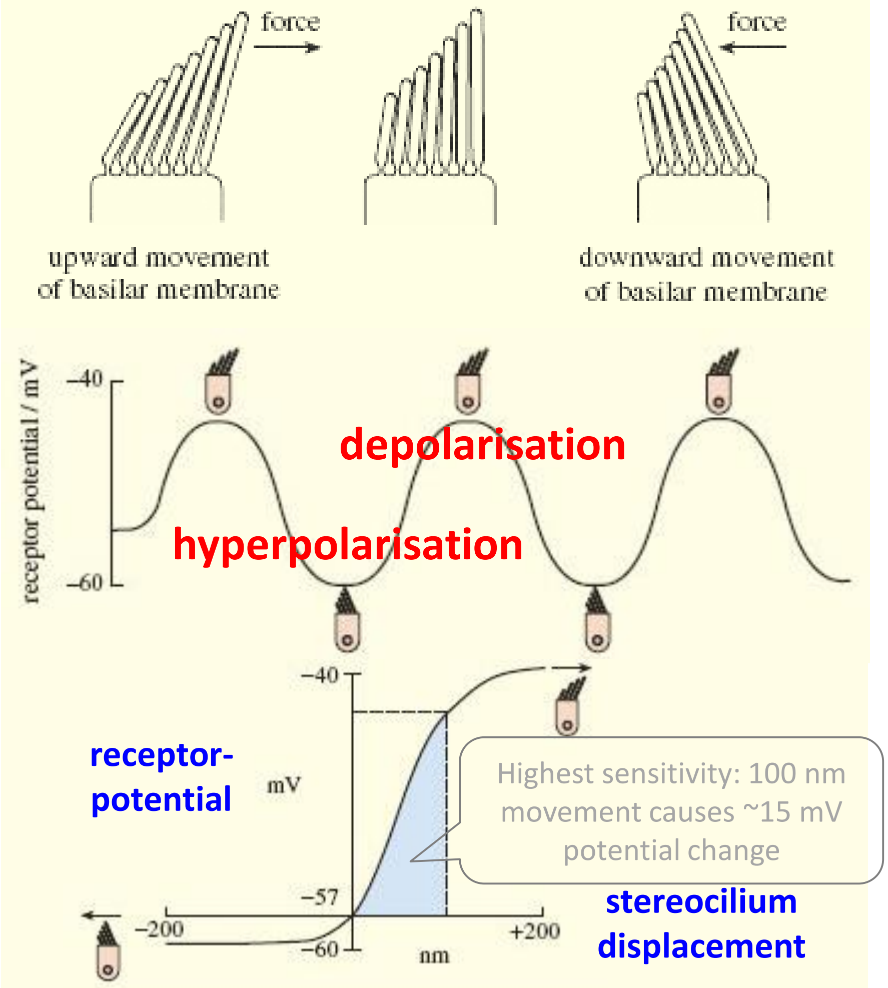
Example: receptor potential

<u>Example for the local change of the membrane potential:</u> hair cells as mechanoreceptors



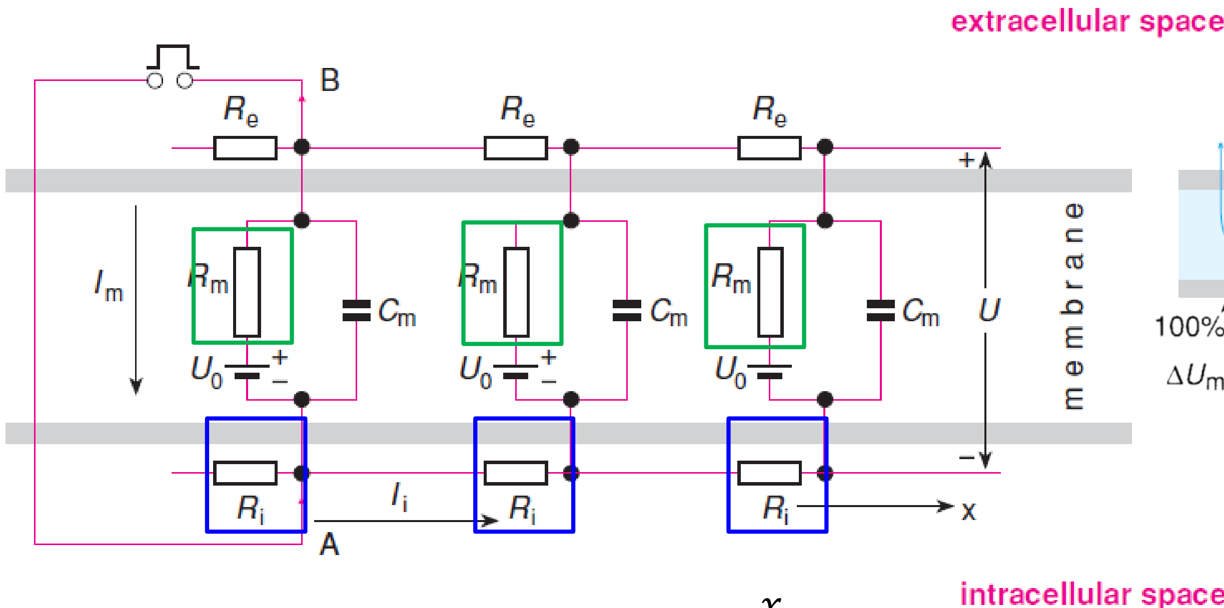


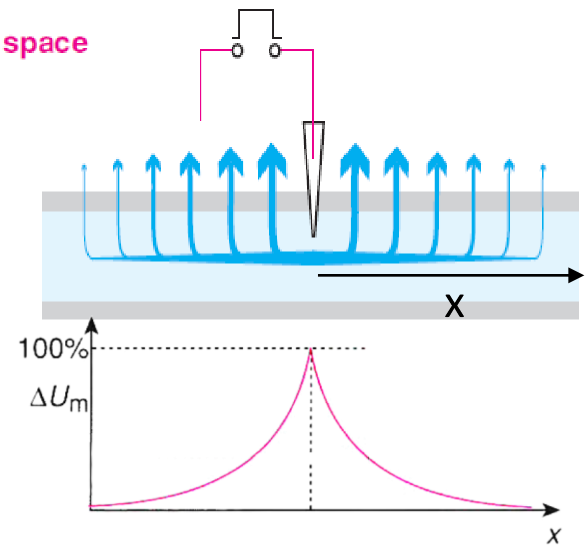
mechanosensitive K^+-channel: K^+ in

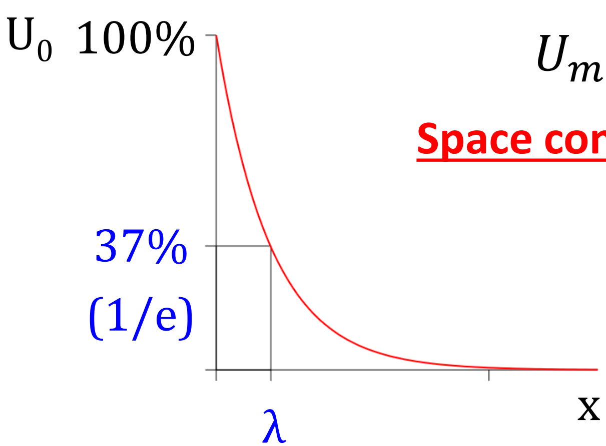


Propagation of a local potential change <u>in space</u>

<u>**Model of a larger membrane section:**</u>







$$U_m(x) = U_0 e^{-\frac{x}{\lambda}}$$

<u>**Space constant:**</u> **lambda, λ** [cm]

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

R_m: transmembrane resistance

R_i: intracellular resistance

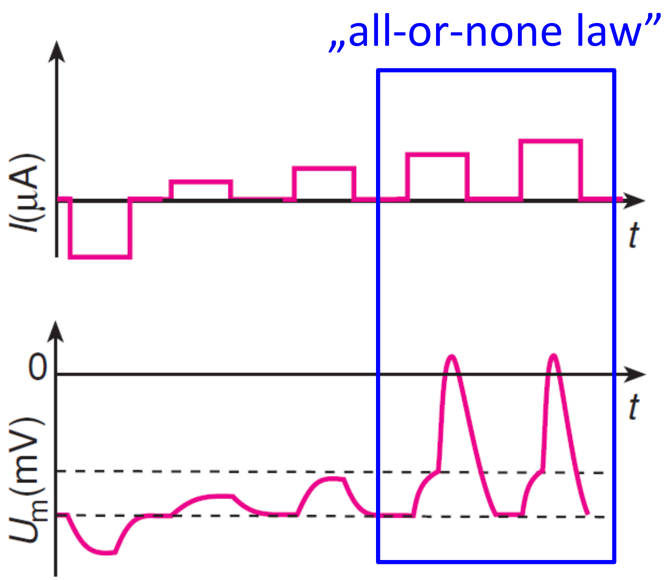
Aim: to increase the value of λ.

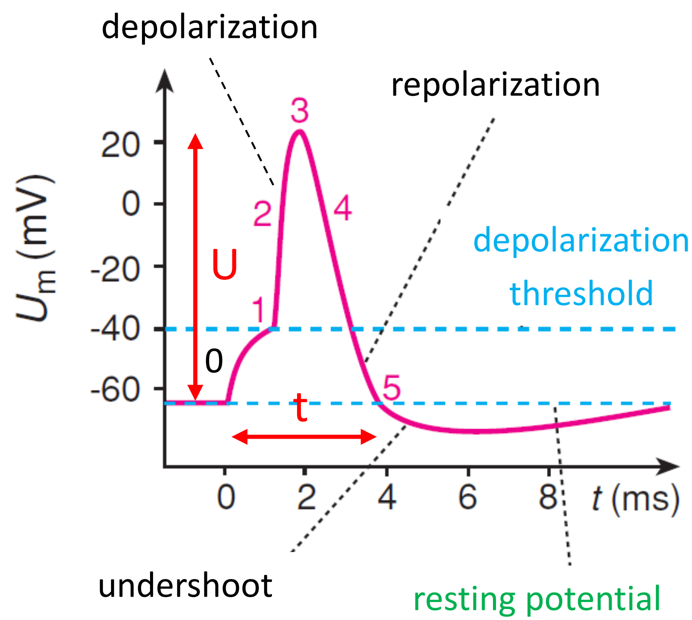
When R_m ↑ or R_i ↓: potential propagation improved.

Example: myelin sheath or larger cell diameter.

Action potential

<u>For stimuli above threshold:</u> generalized change of the membrane potential





t ~ 1-5 ms

(skeletal muscle and neuron)

U ~ 100 mV

0: local change of membrane potential

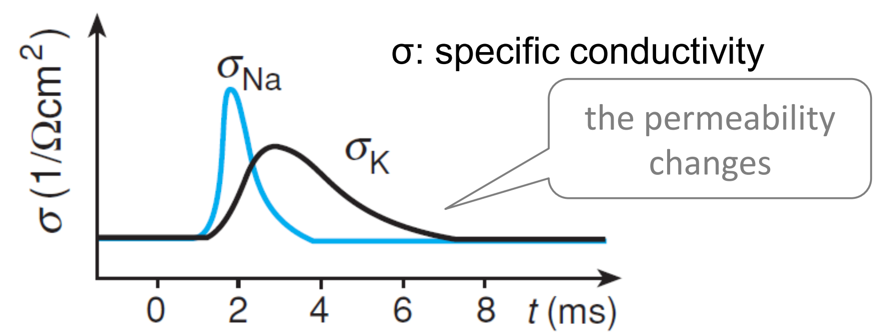
1: **volt. gated Na^+** ch. open (Na^+: **in**)

2: **volt. gated K^+** ch. open (K^+: **out**)

3: **Na^+** ch. **inactivation** (partial)

4: Na^+ channel closure

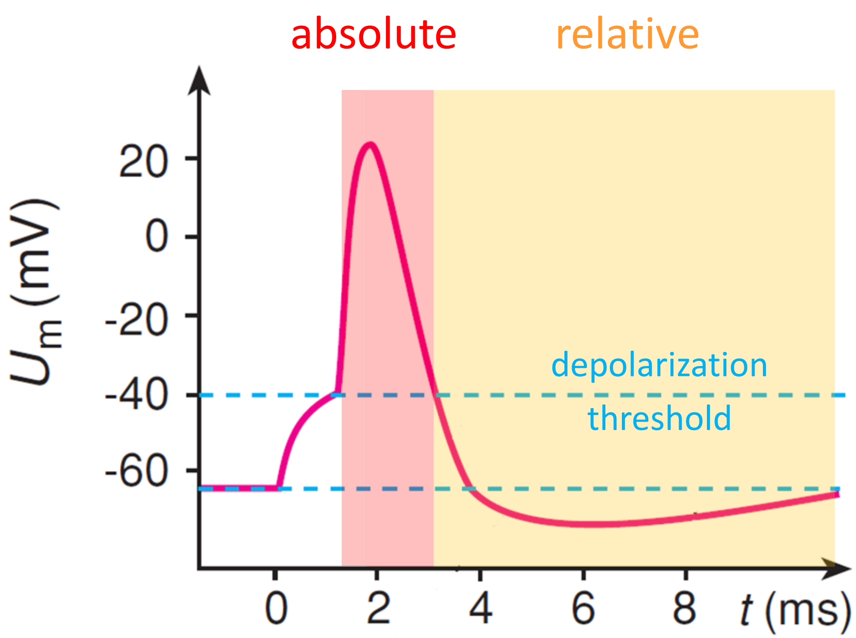
5: **K^+** channel **closure** (delayed)



Properties of the action potential

Unaltered ion concentration: the transported ions diffuse away far from the membrane. During the AP only the permeability changes (GHK).

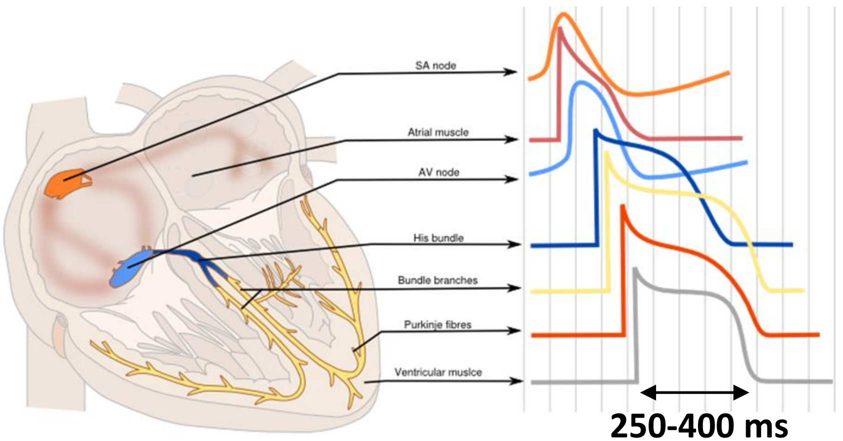
Refractory period: the cell is not excitable

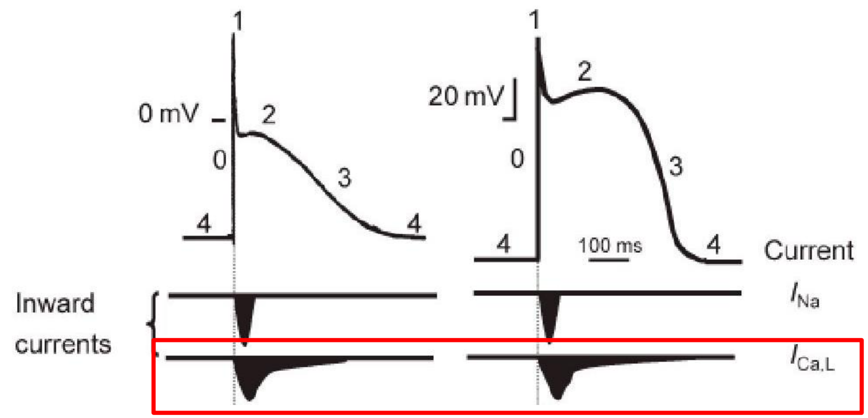


- **absolute:** voltage-gated Na^+ channels are inactivated
- **relative:** AP with supra-threshold stimulus

prevents the backpropagation of AP

Special AP: e.g. ventricular cardiomyocytes



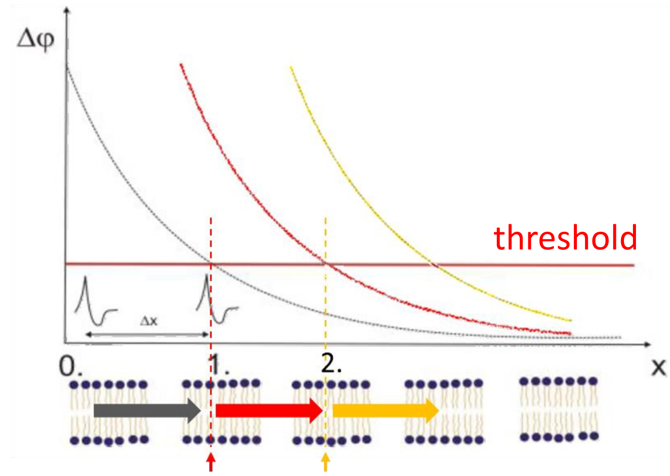


- **voltage-gated Ca^{2+} channels**

The propagation of the action potential

Properties:

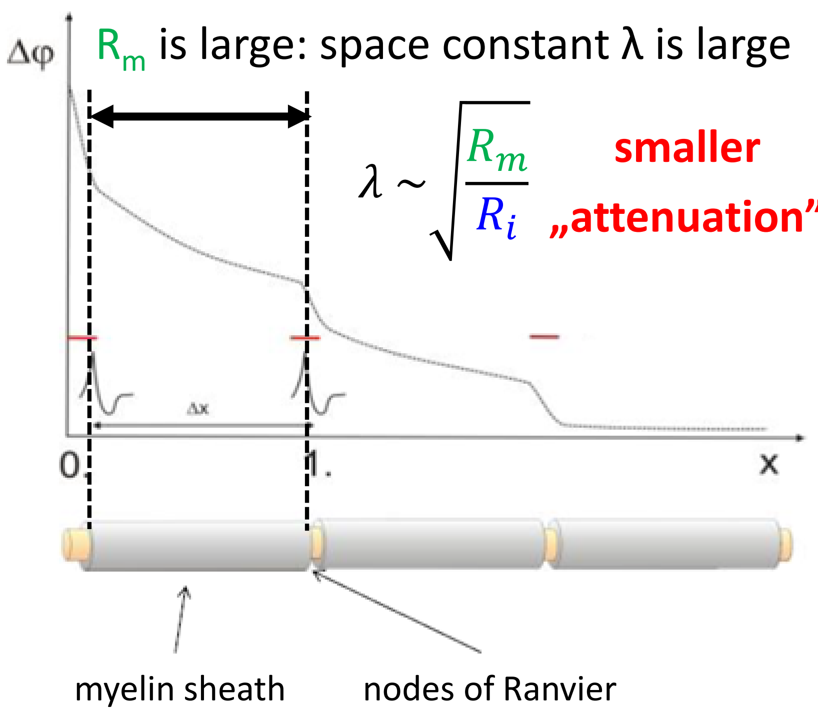
- AP shape is independent from stimulus
- propagates far without attenuation
- much faster than hormonal response



R_m is large: space constant λ is large

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

smaller „attenuation”



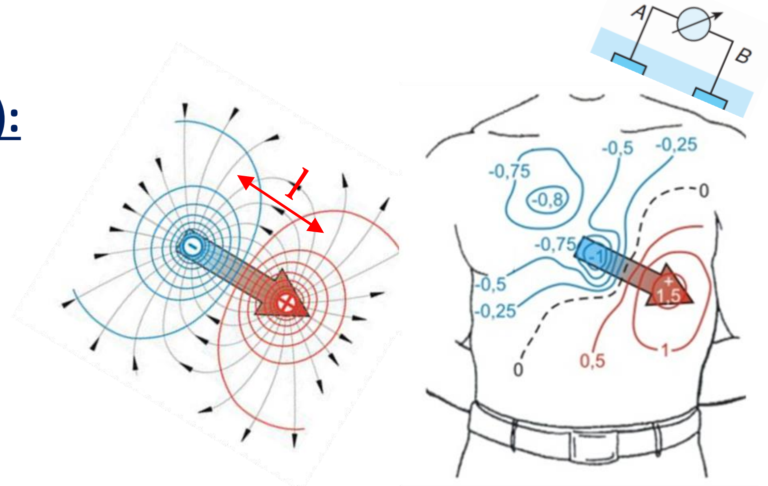
fiber	diameter (μm)	Speed (m/s)
α	15	70-120
β	8	30-70
γ	5	15-30
δ	<3	12-30
No sheath	<1	0.5-2

Medical application of bioelectric phenomena

Electric signals on the body surface (diagnostics):

- Electrocardiography (EKG)
- Electroencephalography (EEG)
- Electromyography (EMG)
- Electrooculography (EOG)
- Electroretinography (ERG)

Origin:
dipole-moment:
$$d = Q \cdot l$$



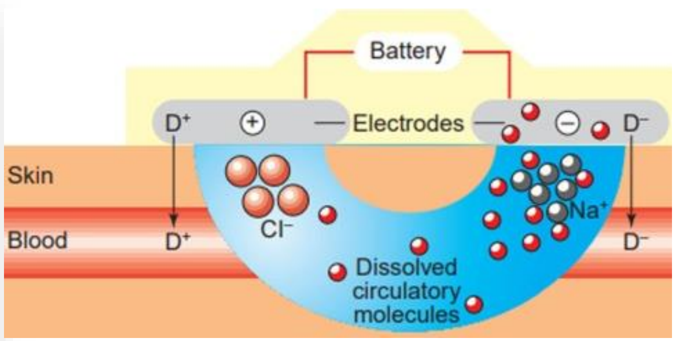
Electric stimulation (therapy):

- Galvanic treatment (DC)
- Iontophoresis (DC)
- HF-thermotherapy (AC)
- Electric surgery (AC)
- Electric stimulus therapy (pulse)
- Defibrillator (pulse)
- Pacemaker (pulse)

Galvanic treatment

- I ~ mA, t ~ 10 min
- improving circulation
- improving metabolism

Iontophoresis

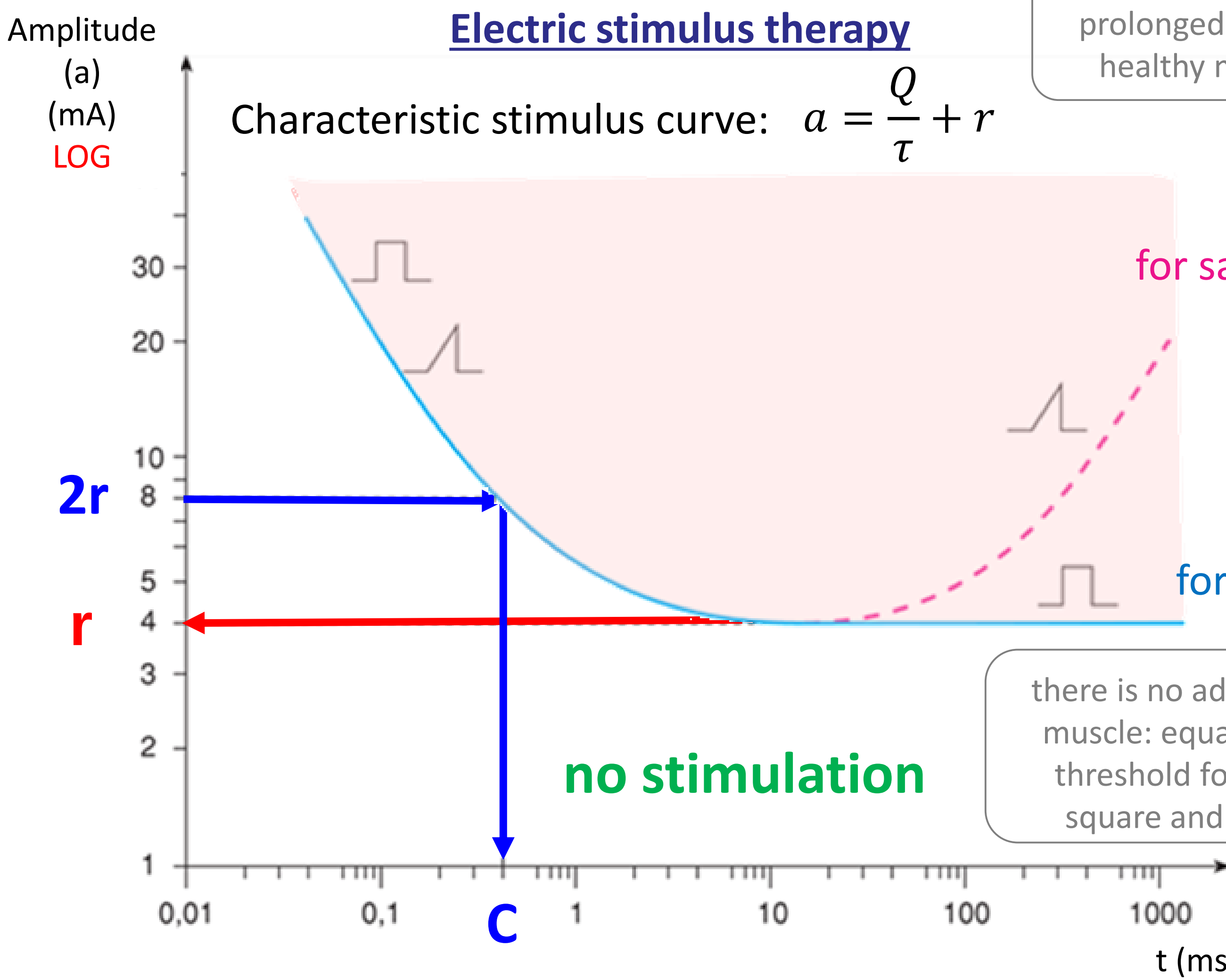


- I ~ mA, t ~ 10 min
- a charged substance is propelled through the dermis into the blood
- the polarity of the electrode should match the charge of the substance
- indifferent „counterion” (e.g. Cl^-)

Pulse therapy

Electric stimulus therapy

Characteristic stimulus curve: $a = \frac{Q}{\tau} + r$



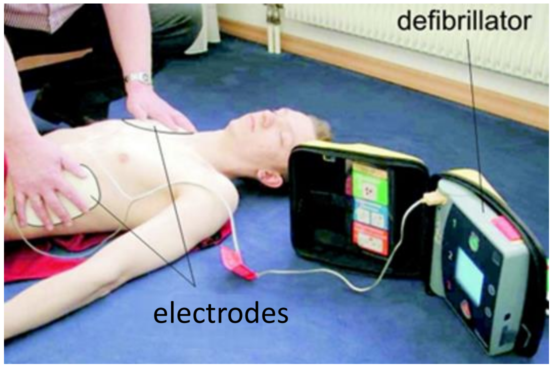
Slowly varying stimulus current: prolonged potential change in healthy muscle: adaptation

there is no adaptation for injured muscle: equally low stimulation threshold for stimulation with square and sawtooth stimuli

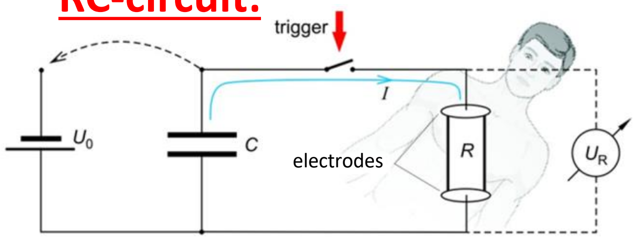
- **rheobase (r):** minimal electric current that elicits (muscle) stimulation
- **chronaxie (C):** time to 2x rheobase

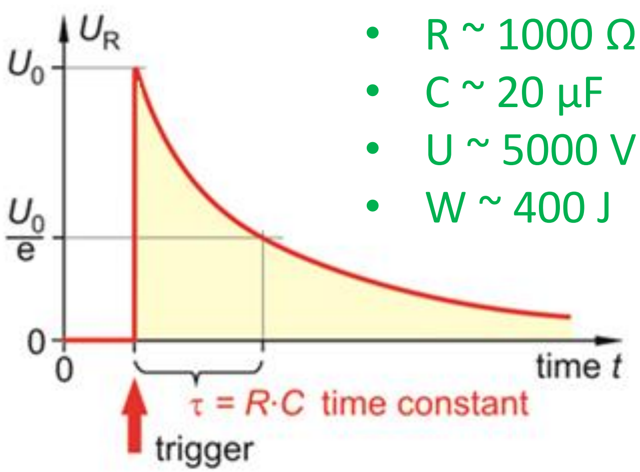
Therapy with electric stimuli

Defibrillator



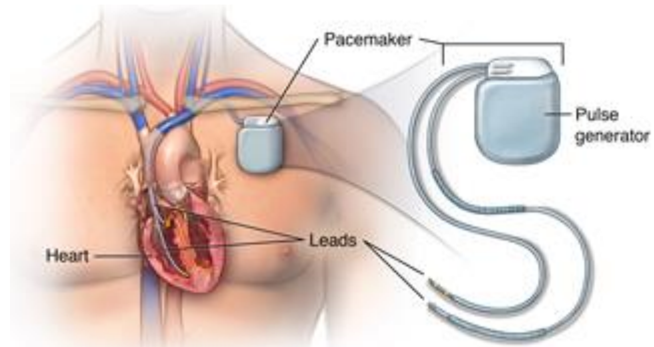
RC-circuit:

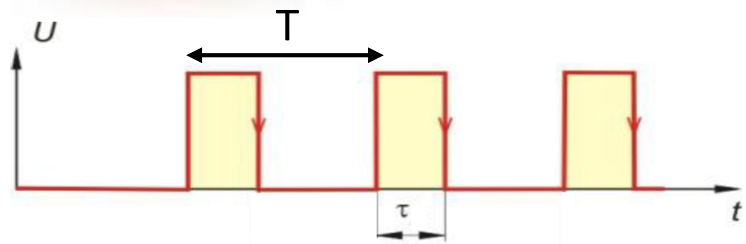




- R ~ 1000 Ω
- C ~ 20 μF
- U ~ 5000 V
- W ~ 400 J

Pacemaker





- τ ~ ms
- T ~ s

- U ~ 1 V
- R ~ 200 Ω
- I ~ 5 mA