# Local change of membrane-potential Laws of sensation

for pharmacy students

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SEMMELWEIS EGYETEM 1769

## Lecture topics

**Topics**

- **Resting membrane potential** (recap)
- **Change of RMP**
  - Graded membrane potential
    - Equivalent circuit model of cell membrane
    - Propagation of potential change
  - Action potential
    - Phases, ion currents
    - Propagation
  - (Voltage clamp, patch clamp)
- **Process of sensation**
  - Receptors
  - Sensory nerve
  - Psychophysical laws

**Related practices**

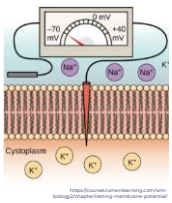
- Diffusion
- ECG
- Sensor
- Audiometry

**Textbook chapters:**

- III/4.3. Changes of membrane potential due to Stimuli below the deplarization threshold
- III/4.4. Membrane potential changes in the excited state: The action potential
- IV/1. General laws of the perception



## Membrane potential

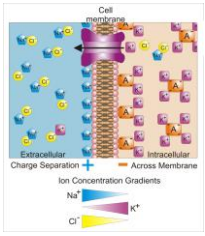



**Transmembrane potential / Membrane voltage / „Resting membrane potential"**

- Electric potential difference between inner and outer surface of the membrane
- Present in all living cell
- Varies among cell types (-30 mV to -90 mV)
- Negative sign: cell interior is negative compared to extracellular space
- Functions:
  - providing power to operate a variety of "molecular devices" embedded in the membrane (cell as battery)
  - in electrically excitable cells such as neurons and muscle cells, it is used for transmitting signals between different parts of a cell



## Membrane potential




By Synaptidude, CC BY 3.0, https://commons.wikimedia.org/w/index.php?curid=21460980

- Two sides of the membrane has different ionic composition

| | Intracellular concentration [mM] | | | Extracellular concentration [mM] | | |
|---|---|---|---|---|---|---|
| Cell type | $Na^+$ | $K^+$ | $Cl^-$ | $Na^+$ | $K^+$ | $Cl^-$ |
| 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 |

- Large phosphate and protein anions inside – p ~ 0
- p is different for the different ions
- Electric and chemical potential difference occurs between the two sides.



## Generation of membrane potential I.

**Model 1**

Presumptions:

- Closed thermodynamic system
- Membrane permable to ions
- Cytoplasm and extracellular space are in thermodynamic **equilibrium** – for **each ion**!
- No net transport of ions
- Thermodynamic force is 0
- Electrochemical potential is the same at the two sides for each type of ion:

$$\mu_{Ci}^{int} - \mu_{Ci}^{ext} = 0$$

$$\mu_0 + RT\ln c_i^{ext} + zF\varphi_i^{ext} = \mu_0 + RT\ln c_i^{int} + zF\varphi_i^{int}$$

$$\varphi_i^{int} - \varphi_i^{ext} = U_0 = \frac{RT}{z_i F}\ln\frac{c_i^{ext}}{c_i^{int}}$$ Nernst equation

*Electric potential of $i^{th}$ ion in equilibrium = equilibrium potential = Electromotive force of a concentration cell of the $i^{th}$ ion*

| | Squid giant axon | Frog muscle |
|---|---|---|
| $U_{measured}$ | -62 mV | -92 mV |
| $U_{ONa+}$ | 47 mV | 46 mV |
| $U_{OK+}$ | -91 mV | -103 mV |
| $U_{OCl-}$ | -56 mV | -88 mV |

**Results:** model failed

- Nerst equation is inadequate to interpret resting potential
- It is not a closed system in equilibrium
- Transport of individual ions is not independent



## Generation of membrane potential III.

**Electrodiffusion model**

- Passive ion diffusion maintains an electric potential difference
- Permeability is different for different ions
- Flux of individual ions ≠ 0
- At rest the transmembrane potential difference is constant →, total electric charge and particle flux must be 0.
- Thus, flux of ions may depend on each other

**Steady state electrodiffusion:**

- Constant electrochemical potential gradient → constant flow of ions → electrically compensate each other → no net charge accumulation
- $Na^+/K^+$ pumps maintains constant ion gradients

**Result:** good agrrement with experimental data

*flux density of $k^{th}$ particle* = $-u_k kT\left(\frac{\Delta c_k}{\Delta x} + c_k\frac{z_k F}{RT}\frac{\Delta\varphi}{\Delta x}\right)$ (*electric potential difference*; *electrochemical potential gradient*; D)

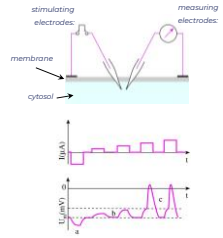
$$\Sigma J_k = 0$$

**Goldman-Hodgkin-Katz equation:**

$$U = \Delta\varphi = \frac{RT}{F}\ln\frac{p_{K^+}[K^+]_{ext} + p_{Na^+}[Na^+]_{ext} + p_{Cl^-}[Cl^+]_{int}}{p_{K^+}[K^+]_{int} + p_{Na^+}[Na^+]_{int} + p_{Cl^-}[Cl^+]_{ext}}$$

| | Squid giant axon | Frog muscle |
|---|---|---|
| $U_{measured}$ | -62 mV | -92 mV |
| $U_{GHK}$ | -61.3 mV | -89.2 mV |

## Electric perturbation of RMP

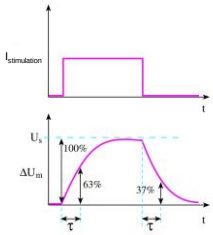



**Experiment**
- Current is driven into the cell
- Square-wave pulses
- Direction and magnitude can be controlled

**Results:**
- **Graded membrane potential**
  - Hyperpolarization / depolarization
  - Size and direction can be controlled
  - Analogue
  - Localized
  - Up to a treshold: RMP changes proportionally
- **Action potential:** reaching a stimulation treshold
  - large, uniform pulse (action potential)



## Electric perturbation of RMP II.



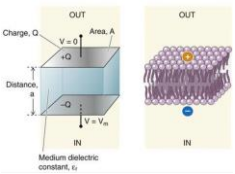

**Depolarizing current below the threshold**
- Square-wave current pulse
- Lower than the depolarization threshold

**Result:**
- Delayed, saturating depolarization
- Decay after stimulation ceases
- Shape: resembling charging and discharging an RC circuit

**Conclusion:**
- Cell membrane could be modelled by a parallel RC circuit.



## Electric circuit model of the membrane




$$E = \frac{U}{d} = \frac{70mV}{5nm} = 1{,}4 \cdot 10^7 \frac{V}{m}$$

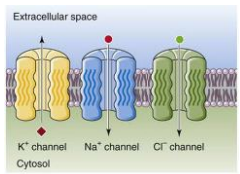
**Membrane as an RC circuit I.**

**Lipid bilayer:**
- Good insulator
- Charges can accumulate on both sides
- Behaves as a capacitor
- Specific capacity
  - constant in time
  - by numbers (for a mammal neuron):
    - $C_m$ = 10 nF/mm²
    - 4*10¹³ charge/cm²
    - $A_{neuron}$ = 0.01-0.1 mm²
    - $C_{neuron}$= 0.1-1 nF

$$C_m = \frac{C}{A} = \frac{Q}{UA}$$



## Electric circuit model of the membrane



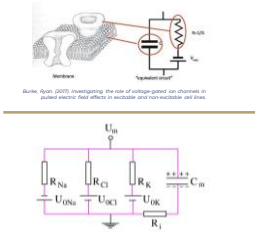

**Membrane as an RC circuit II.**

**Ion channels:**
- Ion-selective permeability → ion currents
- Behave as resistors
- Ion-specific conductivities
  - Constant in resting state
  - Changes upon excitation
  - $G_K$ / $G_{Na}$ / $G_{Cl}$ = 1 / 0.04 / 0.45 (in brain neurons)

$$R_i = \frac{1}{G_i} \qquad \frac{1}{R_{total}} = \sum_{i=1}^{n} \frac{1}{R_i}$$



## Electric circuit model of the membrane




Burke, Ryan. (2017). Investigating the role of voltage-gated ion channels in pulsed electric field effects in excitable and non-excitable cell lines.

**Equivalent circuit model**
- Parallel RC circuit
- For each ion type:
  - permeability → resistance (R=1/G)
  - equilibrium potential → electromotive force ($U_0$)

$$U_{0i} = \frac{RT}{z_i F} \ln \frac{c_i^{ext}}{c_i^{int}}$$ *Nerst equation*

*Conductive current of the i-th ion*

$$I_j = \frac{U_m - U_{0i}}{R_i}$$

*capacitive current*

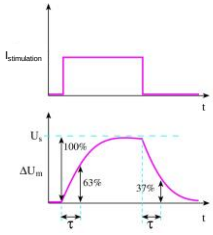
$$I_c = C_m \frac{\Delta U_m}{\Delta t}$$

**GHK:** total charge flux =0

$$\Sigma I = I_c + \Sigma I_j - I_{stimulation} = 0$$



## Electric perturbation of RMP III.




**Changes of membrane potential upon stimulation**
- (1-exponential) time course:

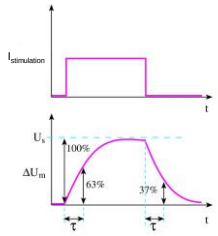
$$U_m(t) = U_S\left[1 - e^{\frac{t}{R_m C_m}}\right]$$

$U_S$: *saturation MP*
$R_m$: *membrane resistance*
$C_m$: *membrane capacitance*
$t$ : *elapsed time*

- Amplitude of change ($\Delta U_m$) is proportianal to stimulus amplitude
- **Time constant of the membrane:** time required to reach 63% of saturation value.

$$\tau = R_m C_m$$

## Electric perturbation of RMP IV.




**Changes of membrane potential following stimulation**

- Exponential decay course:

$$U_m(t) = U_s \cdot e^{-\frac{t}{R_m C_m}}$$

$U_S$: saturation MP
$R_m$: membrane resistance
$C_m$: membrane capacitance
$t$ : elapsed time

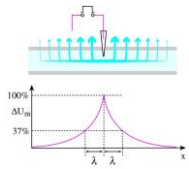
- **Time constant of the membrane:** time required to reach 37% ($U_s$/e) of saturation value.

$$\tau = R_m C_m$$

- Actual membrane potential during stimulation depends on:
  - time constant
  - size and direction of stimulation.



## Propagation of a potential change




**Change of MP:** decreases exponentially with distance

$$U_m(x) - U_m(x_0) = e^{-\frac{x}{\lambda}}$$

space constant of the membrane

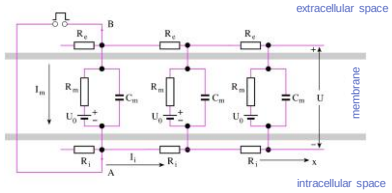
$$\lambda \propto \sqrt{\frac{R_m}{R_i}}$$

**Space constant of membrane:** distance in which the maximal value of the induced potential change decreases to its e-th level (37%)



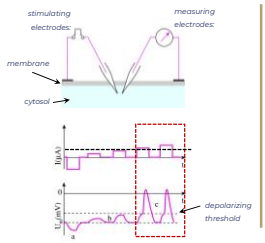
## Propagation of a potential change

**Model of a larger membrane section:**






## Action potential

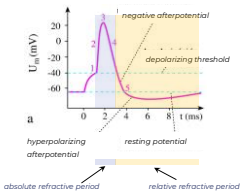



**Action potential:**

- Sudden, fast, transitory and propagating change of the RMP
- Only in excitable cells: neurons, muscle cells
- Trigger: RMP exceeding the depolarization threshold
- Digital: „all or nothing" response
- Characteristic time-dependent shape
- Does not depend on the size or duration of trigger pulse
- few ms duration; cca. 100 mV amplitude
- Propagates along the cell/fibre unidirectionally



## Action potential




**Phases:**

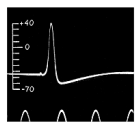
#1 **hypopolarization** (slow, up to threshold)
#2 **depolarization phase** (rapid)
#3 **peak potential**
#4 **repolarization** (rapid)
#5 **hyperpolarizing afterpotential**

- Shape, time course, peak potential: cell-type dependent

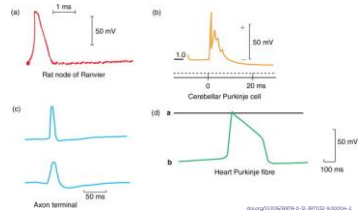
- Depolarizing threshold: changes during AP
  - **Absolute refractive period**: ∞ large threshold, no excitation; around peak potential
  - **Relative refractive period:** higher excitation threshold, mainly during hyperpolarization



## Action potential - examples

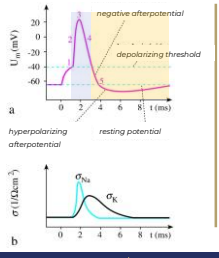



The first intracellular recording of an action potential, from squid axon. Time calibration, 2 ms.
Hodgkin, A. L. & Huxley, A. F. *Nature* 144, 710–712 (1939).



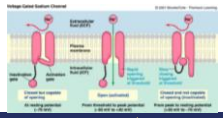

doi.org/10.1016/B978-0-12-397032-9.00004-2

## Action potential – ionic currents



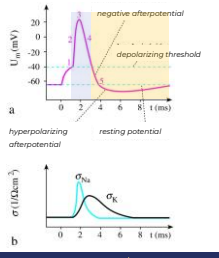

**Sodium channels**

- **Deactivated state** at RMP
- **Activated state:** reaching depolarizing threshold → voltage gated $Na^+$-channels open → $Na^+$ influx → depolarization → more $Na^+$-channels open → further depolarization (+ feedback, Hogdkin cycle)
- **Inactivated state:** reached shortly after opening → channels closed → absolute refractory period





## Action potential – ionic currents




**Potassium channels**

- **Deactivated state:** at RMP
- **Activated state:** reaching depolarizing threshold → voltage gated $K^+$-channels open (delayed kinetics) → $K^+$ outflux → repolarization
- **Inactivated state:** channels close slower → relatively large K+ permeability → transient hyperpolarization → relative refractory period

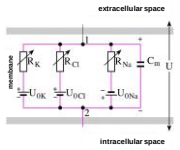
**$Na^+$ / $K^+$ ATPase**

- Restores cytosolic concentration of the cations
- 3 $Na^+$ out / 2 $K^+$ in



## Action potential – modelled

**Modified equivalent circuit model (Hodgkin)**

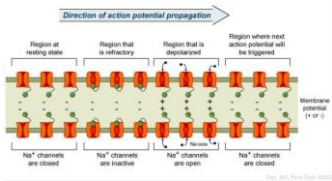



- Permeabilities of ions are not constant → nor the resistances are
- Conductivity depends on membrane potential
- Conductivities can be measured by voltage-clamp technique
- G(U), functions were determined
- Substituted into the modell AP function could be calculated
- Showed good agreement with experimental data.



## Action potential – propagation

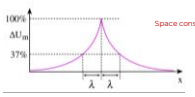
**Propagation of AP along non-myelinated axon(al region)**




- Exponential decay of peak potential in space – determined by **space constant**
- Large enough depolarizations in nearby regions to exceed threshold → AP is generated.
- $Na^+$ channels become inactive in previousely activated areas → absolute refracter period.
- By the time the refractory periods cease depolarisation wave travelled far enough to avoid re-excitation.
- Unidirectional propagation
- v=1-30 m/s (mammal neurons)



## Action potential – propagation II.

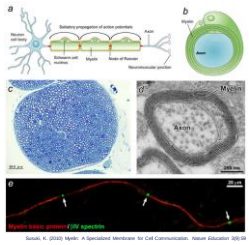



Space constant:

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

How is it possible to increase the speed of propagation?

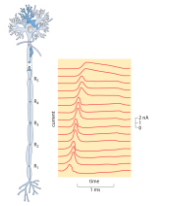
- Increase $R_m$ : **myelinization** (decreases $C_m$ and thus the time constant as well)
- Decrease $R_i$ : enlargement of cellular (axonal) diameter – non advantageous



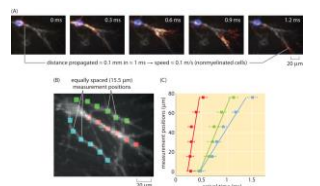

Susuki, K. (2013). Myelin: A Specialized Membrane for Cell Communication. Nature Education 3(9):59



## Action potential – propagation III.



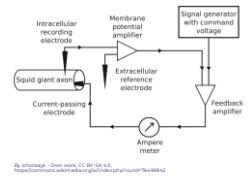
Measurement of the propagation of a nervous impulse. (Adapted from A. F. Huxley and R. Stampfli, J. Physiol. 108:315, 1949.)



Optical measurement of action potential speed. (A) Series of images of the fluorescence in the cell as a function of time. (B) A series of equally spaced (15 μm) measurement points along three different processes are used to measure the arrival time of a propagating action potential. (C) Arrival times for the three processes shown in part (B).

## Voltage clamp

**Experimental setup**




By smonaya - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=76498842

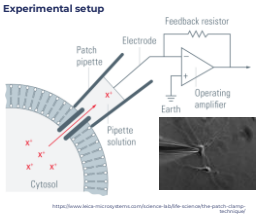
**Working principle**

- MP kept constant regardless transmembrane ionic currents
- Intracellular electrode #1: records actual MP
- Intracellular electrode #2:
  - conducts current to set MP to command U value → MP is clamped
  - Current amplitude is equal to membrane current (I)
- Channels can be blocked ($Na^+$: tetradotoxin; $K^+$: tetra-ethyl ammonium) → specific ion currents can be calculated
- MP-dependent conductivity of ions can be determined



## Patch clamp

**Experimental setup**




https://www.leica-microsystems.com/science-lab/life-science/the-patch-clamp-technique/

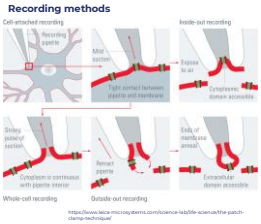
**Working principle**

- Glass micropipette ($d_{tip}$=0.5-1 µm) attached to a cell
- Large electrical and mechanical resistance contact („giga-seal")
- Cell Attached Patch (CAP)
- Membrane patch with 1-few ion channels
- Measuring electrode in the electrolyte inside pipette
- Voltage-clamp setup → currents (pA-s) are measured
- Single opening – unitary current jump
- $10^6$-$10^7$ ions / few ms; conductance: ~pS
- **Applications:**
  - Ion-channel studies (drug effects; receptor-ligand binding; kinetics, activation, inhibition, etc.)



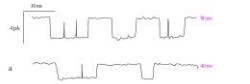
## Patch clamp

**Recording methods**

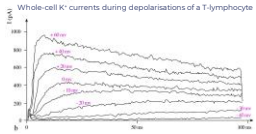



https://www.leica-microsystems.com/science-lab/life-science/the-patch-clamp-technique/

Single channel currents (human blood lymphocyte, CAP)

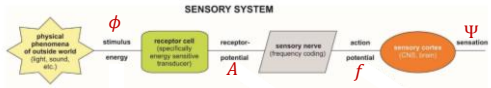


Whole-cell $K^+$ currents during depolarisations of a T-lymphocyte





## Process of sensation



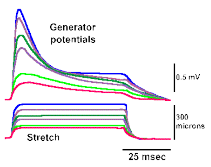

**Modality:** types of sensation, eg.: vision, hearing, olfaction, taste, touch, pain, thirst, muscle fatigue.
**Quality:** psychophysical sensation given by modality, eg.: loudness, colour shade
**Quantity:** degrees of a quality, eg.: son-loudness; brightness
**Perception:** interpretation of the sensation, eg.:. experience of vision, voice recognition



## Receptors

**Receptor cell**

- Transducer: converts physical/chamical stimulus energy into receptorpotential
- Receptor potencial (generator potential): stimulus-triggered, local change of MP
- Amplitude coding: amplitude depends on stimulus intensity
- Specifically energy sensitive
- Stimulus energy – wide range
- Dynamic compression/expansion: weak stimuli results in relatively larger/smaller change of MP than strong stimuli
- Adaptation: constant stimulus intensity – decreasing receptorpotential




Graded responses of a muscle spindle receptor to stretch. Graded stretches are indicated by the stretch monitor in the lower traces; graded generator potentials are shown in the upper traces
Ottoson D and Shepherd GM: *Cold Spring Harbor Symp Quant Biol* 30:105-114, 1965.



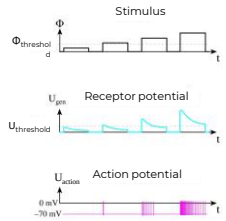
## Receptors

| Classification | Receptor cell/group | What does it sense? | Example |
|---|---|---|---|
| According to stimulus | Photoreceptor | Light (J; λ) | Retina |
| | Chemoreceptor | Chemical substances (concentration) | Taste buds (tongue); olfactory receptors (nose) |
| | Thermoreceptor | Δt | Skin thermoreceptors |
| | Mechanoreceptor | p (touch) | Skin, hair |
| | Baroreceptor | p | Blood vessel wall |
| According to location | Exteroreceptor | External conditions | Eye, ear, nose, tongue, skin |
| | Interoreceptor | Internal conditions | Blood vessel wall baroreceptors, pain sensors |
| | Proprioceptor | Position of body parts | Muscle, joint |
| According to complexity | General sensory organs | Individual or grouped receptor cells | Hat, touch |
| | Specialized sensory organs | Complex sensory organs (large number of receptor cells) | Eye, ear, nose, tongue |

## Sensory nerve

**Nerve cell**

- Attached to receptor cell.
- Answers with AP when receptor potential exceeds depolarisation threshold.
- AP propgates fast alogside nerve fiber to reach the sensory cortex.
- Frequency coding: frequency of AP proportional to the size of receptor potential.






## Psychophysical laws – I.

**Weber-Fechner law**

$$\psi = a \cdot log_b \frac{\Phi}{\Phi_0}$$

*ψ: strenght of sensation*
*a and b: constants*
*Φ: absolute stimulus intensity*
*$\Phi_0$: absolute threshold stimulus*

- Sensation is proportional to the logarithm of relative stimulus intensity ($\Phi/\Phi_0$)
- Very limited validity

**Stevens law**

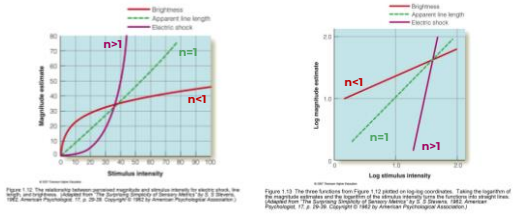
$$\psi = a \cdot \left[\frac{\Phi}{\Phi_0}\right]^n$$

*a and n: constants*

- Sensation is proportional to the power of relative stimulus intensity ($\Phi/\Phi_0$)
- Valid over wide ranges of stimulus intensities
- n<1 → compressive function (pl. loudness: n=0,3)
- n>1 → expansive function (pl. electric schock: n=3,5)



## Psychophysical laws – II.






## Thanks for your attention!

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