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

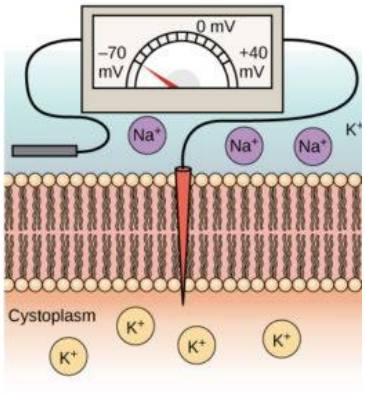
Related practices

- 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

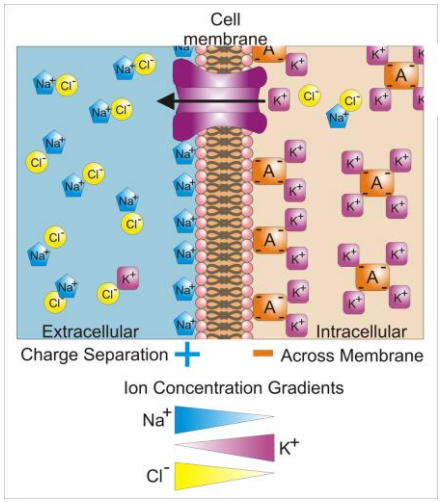


https://courses.lumenlearning.com/wm-biology2/chapter/resting-membrane-potential/

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=21460910

- 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:

$$\mu_{e,i}^{int} - \mu_{e,i}^{ext} = 0$$

$$\mu_0 + RTlnc_i^{ext} + zF\varphi_i^{ext} = \mu_0 + RTlnc_i^{int} + zF\varphi_i^{int}$$

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

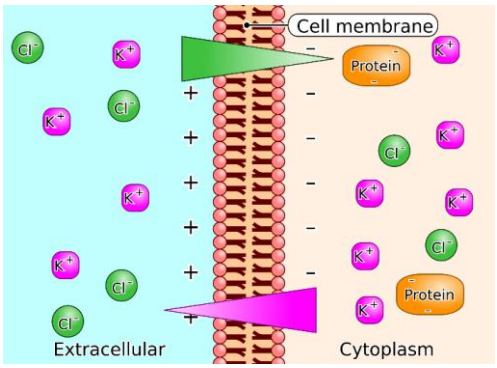
Electric potential of i^{th} ion in equilibrium = equilibrium potential = Electromotive force of a concentration cell of the i^{th} ion

	Squid giant axon	Frog muscle
$U_{measured}$	-62 mV	-92 mV
U_{0Na+}	47 mV	46 mV
U_{0K+}	-91 mV	-103 mV
U_{0Cl-}	-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 II.



Donnan equilibrium (Gibbs-Donnan effect)

- Closed thermodynamic system
- Presence of nonpermeable charged particles in one side of a membrane leads to noneven distribution of ions.
- Membrane is permeable only to K^+ and Cl^-
- Electroneutrality at the two sides,
- Electrochemical potential is the same at the two sides for each type of ion.

Donnan potential:

- Equilibrium potential difference due to ionic concentration differences (determinded by the presence of nondiffusible anions)
- Typical value: U_{Donnan} ~ -14 mV → relatively small contribution to membrane potential.

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 elecrodiffusion:

- 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 kth particle: *electric potential difference*

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

D — *electrochemical potential gradient*

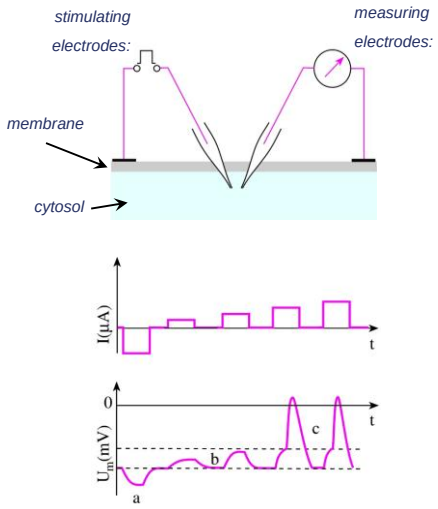
$$\Sigma J_k = 0$$

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



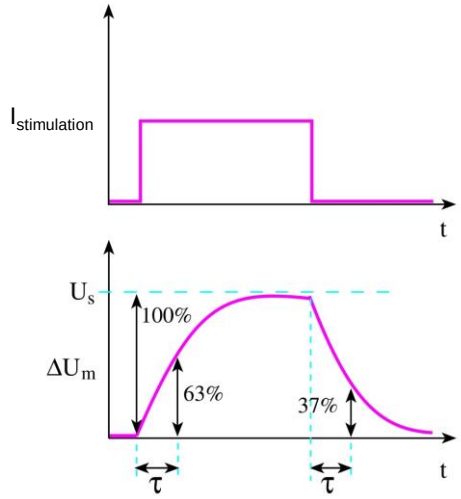
Experiment

- 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

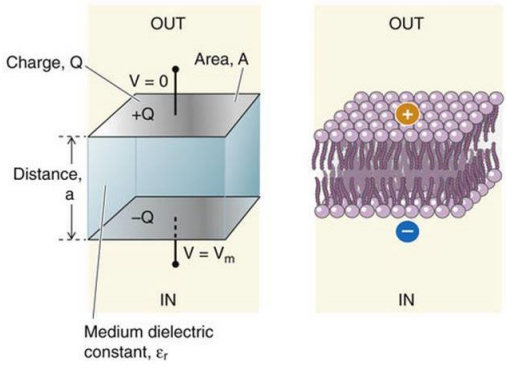
Result:

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

Conclusion:

- Cell membrane could be modelled by a parallel BC circuit.

Electric circuit model of the membrane

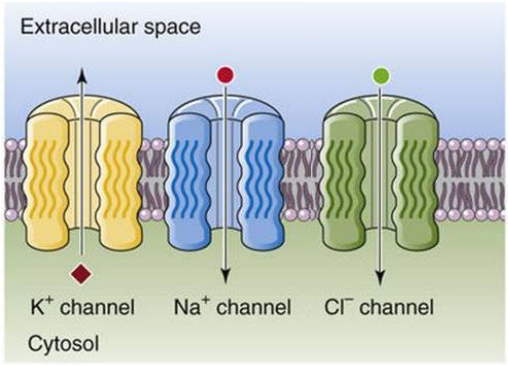


Membrane as an RC circuit I.

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/mm^2
    - 4*10^{11} charge/cm^2
    - A_{neuron} = 0.01-0.1 mm^2
    - C_{neuron}= 0.1-1 nF

Electric circuit model of the membrane

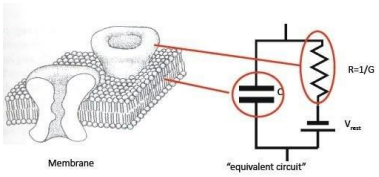


Membrane as an RC circuit II.

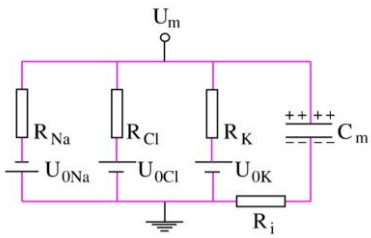
Ion channels:

- 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)

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

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

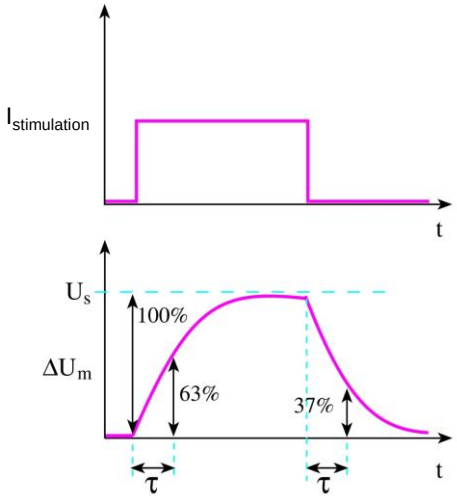
capacitive current

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

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:

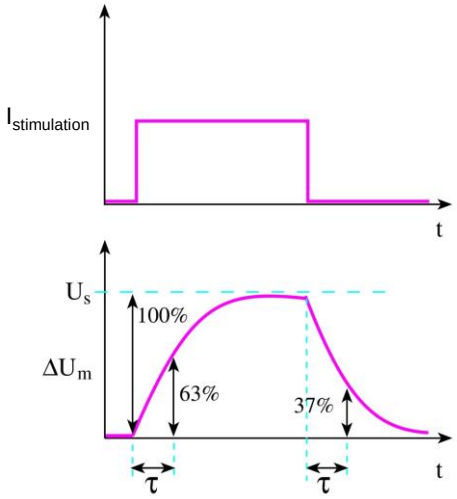
$$U_m(t) = U_S\left[1 - e^{\frac{t}{R_m C_m}}\right]$$

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

- Amplitude of change (ΔU_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*

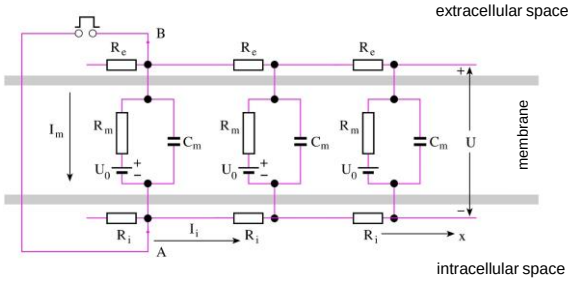
- **Time constant of the membrane:** time required to reach 37% (U_S/e) of saturation value.

$$\tau = R_m C_m$$

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

Propagation of a potential change

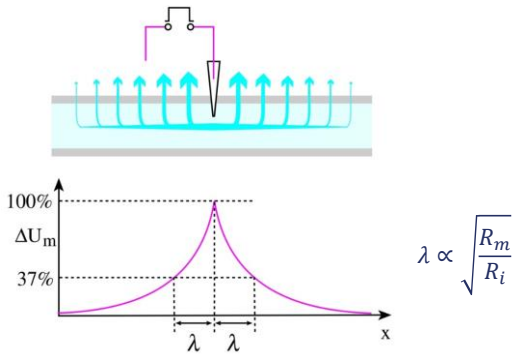
Model of a larger membrane section:



Change of MP: decreases exponentially with distance

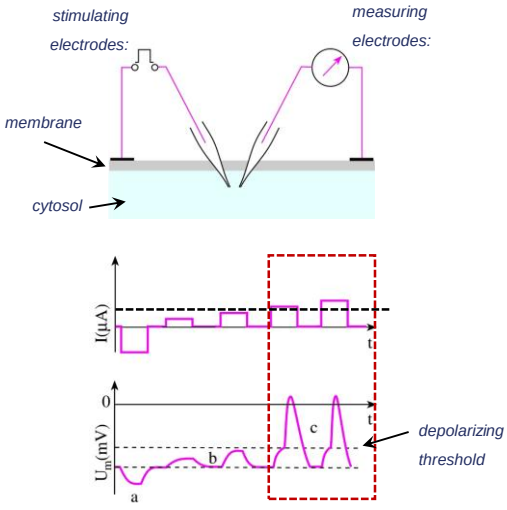
$$U_m(x) - U_m(x_0) = e^{-\frac{x}{\lambda}}$$

space constant of the membrane



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

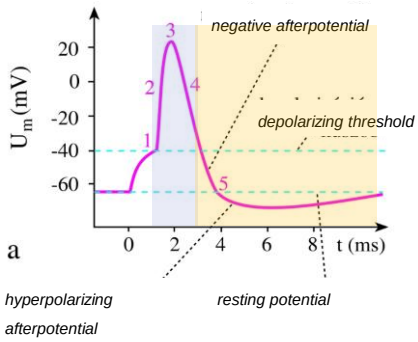
Action potential



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
- Propagates along the cell/fibre

Action potential



Phases:

#1 **hypopolarization** (slow, up to threshold)

#2 **depolarization phase** (rapid)

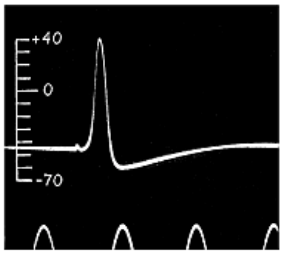
#3 **peak potential**

#4 **repolarization** (rapid)

#5 **hyperpolarizing afterpotential**

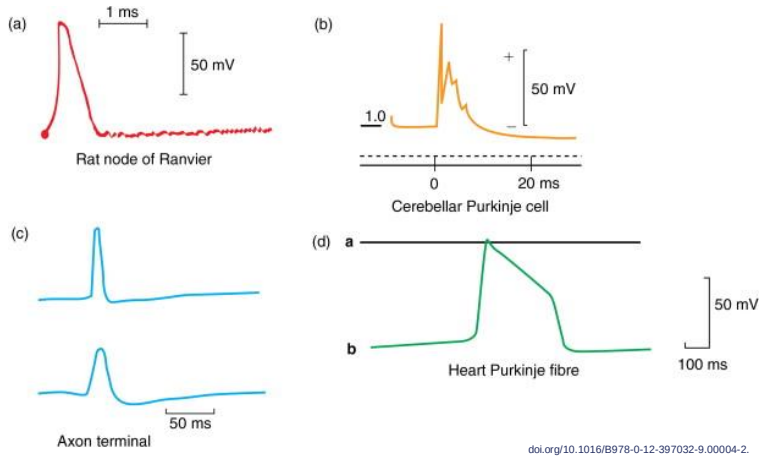
- Shape, time course, peak potential: cell-type dependent
- Depolarizing threshold: changes during AP
  - **Absolute refractive period**: ∞ large threshold, no excitation; around peak potential
  - **Relative refractive period:** higher excitation threshold, mainly during hyperpolarization

Action potential - examples



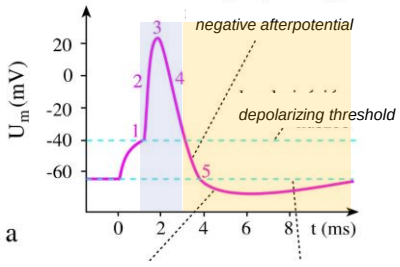
The first intracellular recording of an action potential, from squid axon. Time calibration, 2 ms.

Hodgkin, A. L. & Huxley, A. F. Nature 144, 710–712 (1939).

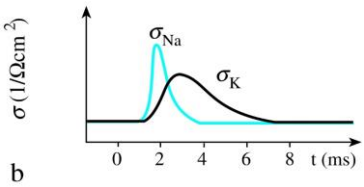


doi.org/10.1016/B978-0-12-397032-9.00004-2.

Action potential – ionic currents



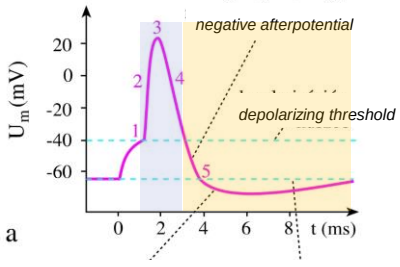
hyperpolarizing afterpotential *resting potential*



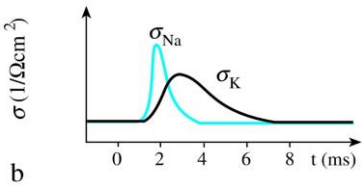
Sodium channels

- **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



hyperpolarizing afterpotential *resting potential*



Potassium channels

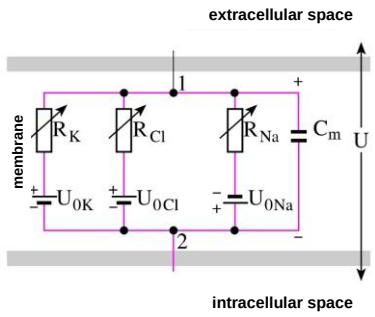
- **Deactivated state:** at RMP
- **Activated state:** reaching depolarizing threshold → voltage gated K^+-channels open (delayed kinetics) → K^+ outflux → repolarization
- **Inactivated state:** channels close slower → relatively large K+ permeability → transient hyperpolarization → realtive refractory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

Na^+ / K^+ ATPase

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

Action potential – modelled

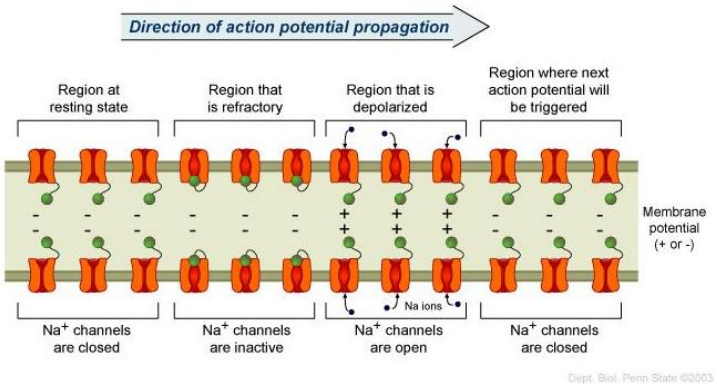
Modified equivalent circuit model (Hodgkin)



- Permeabilities of ions are <u>not constant</u> → nor the resistances are
- Conductivity depends on membrane potential
- Conductivities can be measured by voltage-clamp technique
- $G(U)_i$ 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 previously activated areas → absolute refracter period.
- By the time the refractory periods cease depolarisation wave trevelled far enough to avoid re-excitation.
- Unidirectional propagation
- v≈1-30 m/s (mammal neurons)

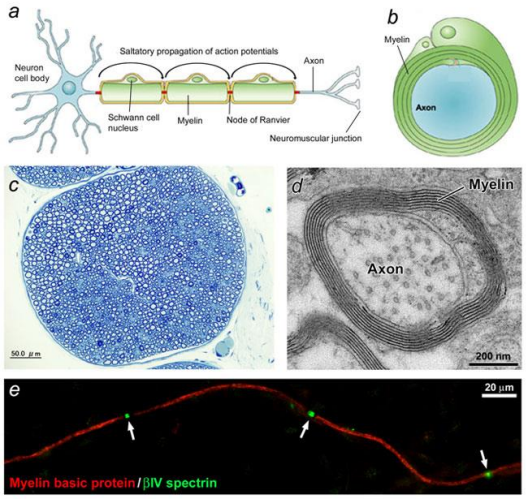
Action potential – propagation II.

Space constant: $\lambda \propto \sqrt{\frac{R_m}{R_i}}$

Time constant: $\tau = R_m C_m$

How is it possible to increase the speed of propagation?

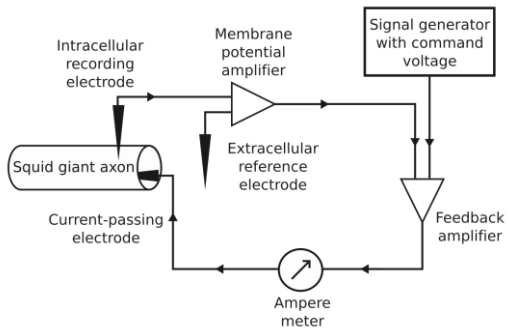
- Increase R_m : **myelinization** (decreases C_m and thus the time constant as well)
- Decrease R_i : enlargement of cellular (axonal) diameter – non advantageous



Susuki, K. (2010) Myelin: A Specialized Membrane for Cell Communication. *Nature Education* 3(9):59

Voltage clamp

Experimental setup

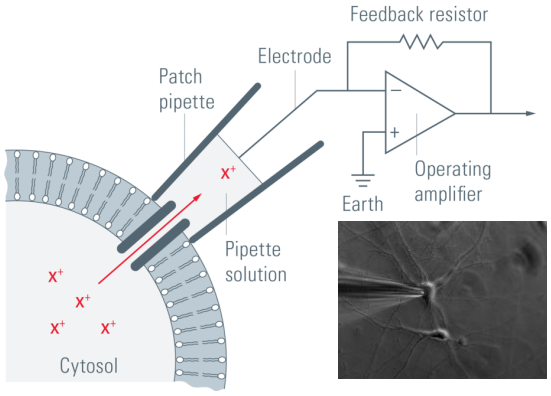


Working principle

- 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^+: terta-ethyl ammonium) → specific ion currents can be calculated
- MP-dependent conductivity of ions can be determined

Patch clamp

Experimental setup



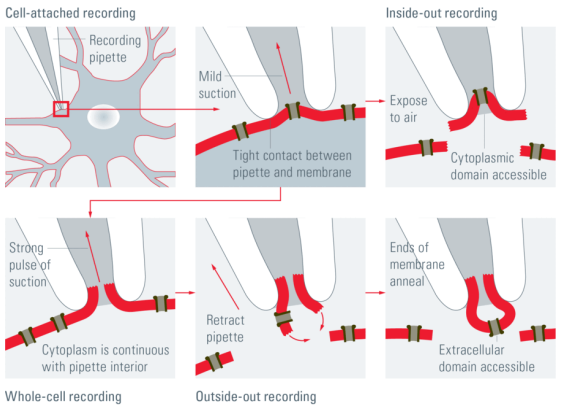
https://www.leica-microsystems.com/science-lab/life-science/the-patch-clamp-technique/

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
- 10^5-10^7 ions / few ms; conductance: ~pS
- **Applications:**
  - Ion-channel studies (drug effects; receptor-ligand binding; kinetics, activation, inhibition, etc.)

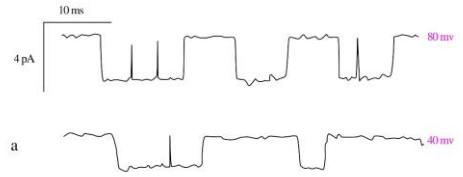
Patch clamp

Recording methods

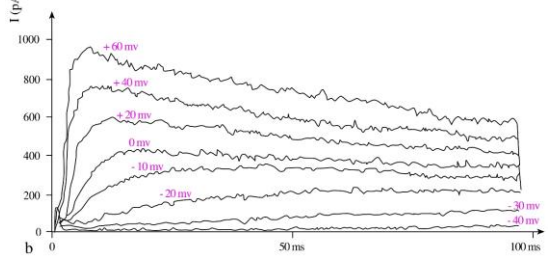


https://www.leica-microsystems.com/science-lab/life-science/the-patch-clamp-technique/

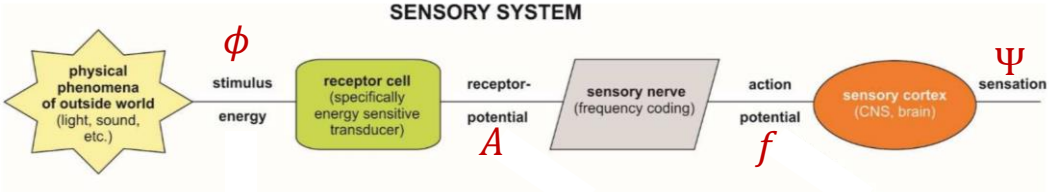
Single channel currents (human blood lymphocyte, CAP)



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

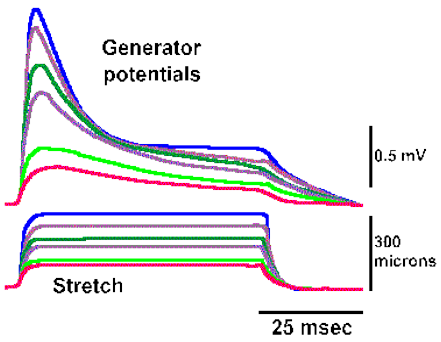
Quantity: degrees of a quality, eg.: son-loudness; brightness

Perception: interpretation of the sensation, eg.:. experience of vision, voice recognition

Receptors

Receptor cell

- Transzducer: converts physical/chamical stimulus energy into receptorpotential
- Recepto rpotencial (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.

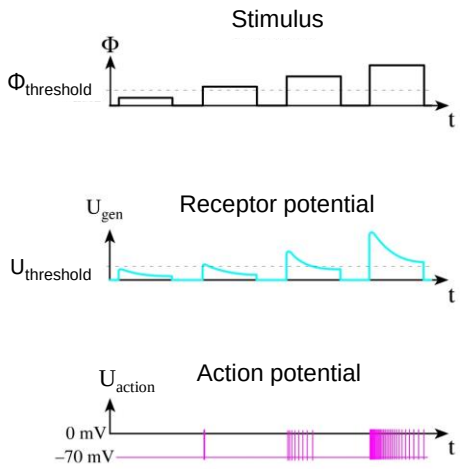
Receptors

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*

Φ_0: *absolute threshold stimulus*

- Sensation is proportional to the logarithm of relative stimulus intensity (Φ/Φ_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 (Φ/Φ_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 – I.

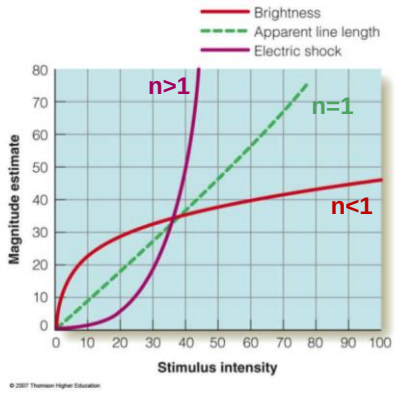


Figure 1.12 The relationship between perceived magnitude and stimulus intensity for electric shock, line length, and brightness. (*Adapted from "The Surprising Simplicity of Sensory Metrics" by S. S Stevens, 1962, American Psychologist, 17, p. 29-39. Copyright © 1962 by American Psychological Association.*)

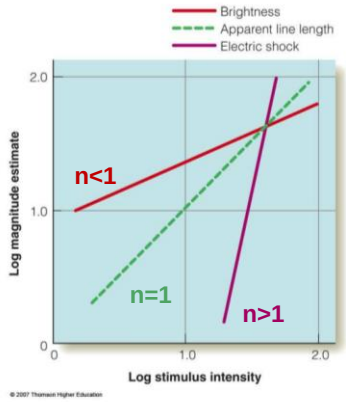


Figure 1.13 The three functions from Figure 1.12 plotted on log-log coordinates. Taking the logarithm of the magnitude estimates and the logarithm of the stimulus intensity turns the functions into straight lines. (*Adapted from "The Surprising Simplicity of Sensory Metrics" by S. S Stevens, 1962, American Psychologist, 17, p. 29-39. Copyright © 1962 by American Psychological Association.*)

Thanks for your attention!

Dr. Tamás Bozó

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