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**SEMMELWEIS
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Development of Complex Curricula for Molecular Bionics and Infobionics Programs within a consortial* framework**

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Consortium members

SEMMELWEIS UNIVERSITY, DIALOG CAMPUS PUBLISHER

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ELECTROPHYSIOLOGICAL METHODS FOR THE STUDY OF THE NERVOUS- AND MUSCULAR-SYSTEM

(Az ideg- és izom-rendszer elektrofiziológiai vizsgálómódszerei)

LECTURE 3

MEMBRANE PROPERTIES, RESTING POTENTIAL

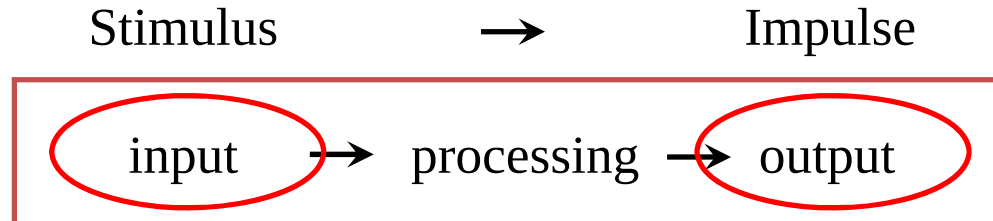
(Membrán tulajdonságok, nyugalmi potenciál)

RICHÁRD CSERCSA, ISTVÁN ULBERT
and GYÖRGY KARMOS

AIMS:

In this lecture, the student will become familiar with the basic electrical properties of a nerve cell. They will learn about the semi-permeable membrane, the ions producing the membrane potential, and the generation of the resting potential.

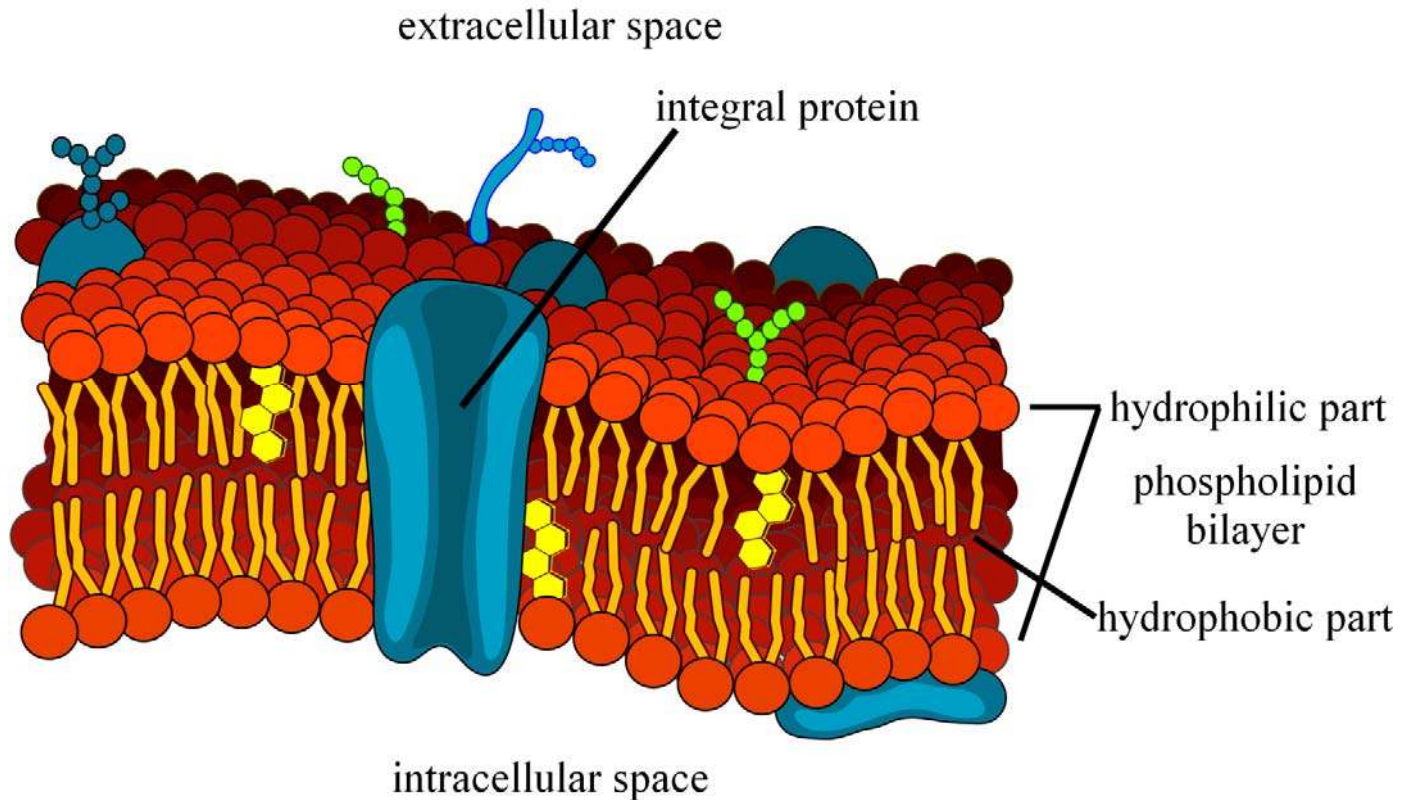
NEURON AS INFORMATION PROCESSING UNIT



During physiological operation, neurons receive input (stimuli) from other neurons. They acquire this information through synapses on their dendrites. Then they process the information. Finally they pass on the output (impulse) to other neurons through synapses on their axons.

The inputs to a cell are called postsynaptic potentials (PSP). They can be excitatory (EPSP) or inhibitory (IPSP). They modify the membrane potential of the cell, thus changing its excitability. EPSPs bring the membrane potential closer to a firing threshold, IPSPs make it go farther. If the threshold is reached, an action potential is generated and that is the output of the cell.

STRUCTURE OF NEURON MEMBRANE



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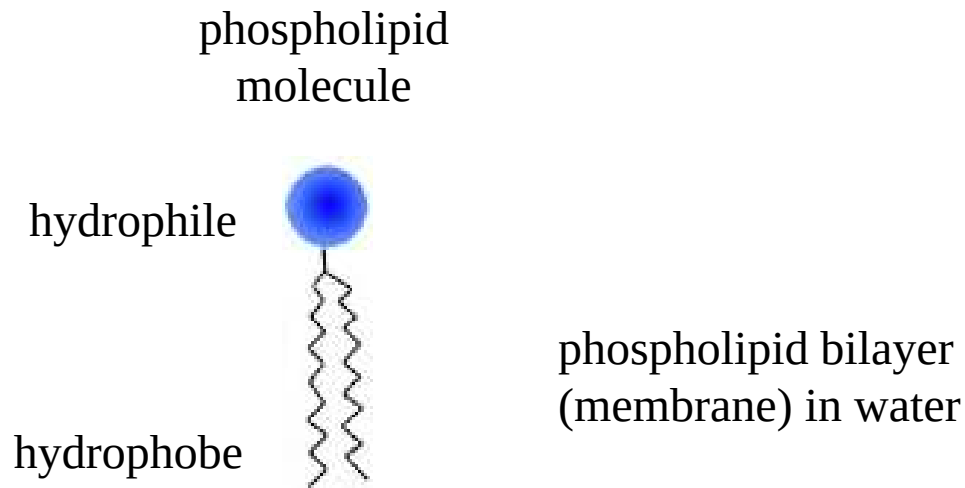
STRUCTURE OF NEURON MEMBRANE

The neuron membrane is a phospholipid bilayer that separates the intracellular and the extracellular fluids. Each phospholipid molecule consists of a hydrophilic and a hydrophobic part. Molecules are organized into layers such that hydrophobic parts are inside the membrane, and hydrophilic parts contact with the external world. This structure makes the membrane not permeable for ions and charged molecules, thus a good dielectric.

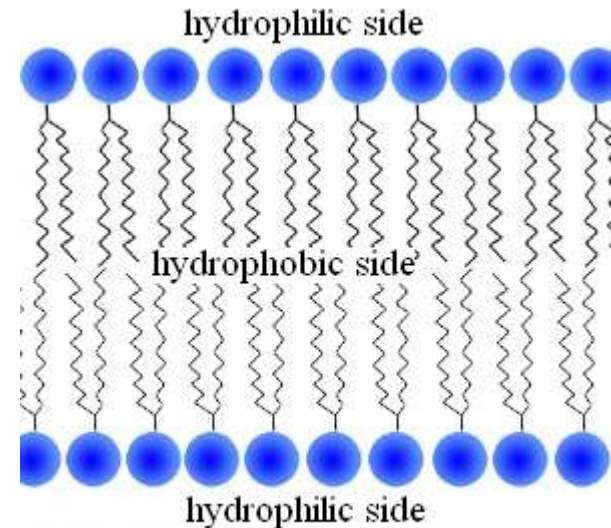
Certain proteins may be embedded in the membrane. They can be peripheral, if they do not span through the whole membrane, or transmembrane, if they reach both sides of the membrane.

These proteins are called ion channels and ion transporters if they can transport ions from one side of the membrane to the other.

BUILDING BLOCKS OF NEURON MEMBRANE



phospholipid bilayer
(membrane) in water



- good dielectric
- not permeable for ions, charged molecules
- not permeable for big molecules
- permeable for water and small, uncharged molecules
- molecules soluble in fat may dissolve in the membrane

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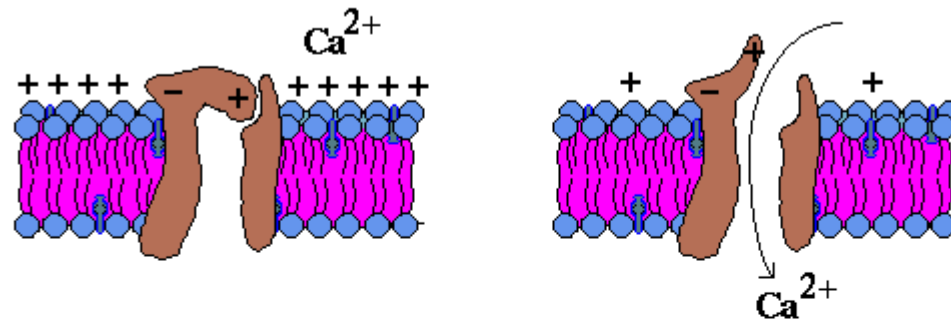
ION CHANNELS

Ions can be transported through the cell membrane passively, without energy investment, or actively, when energy is needed for the transport. The necessary energy comes from adenosine triphosphate (ATP) dephosphorylation.

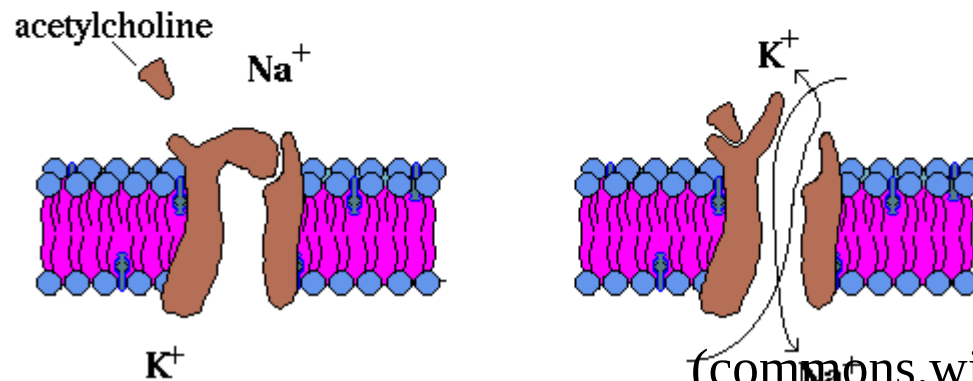
Ion channels can be closed, when they are in a conformation that they cannot transport ions, or open. Ion channels can be ligand gated or voltage gated, depending on the way they can become open. Ligand gated channels require certain molecules attached to them in order to open, while for voltage gated channels, a certain potential difference between the two sides of the membrane is necessary.

PROTEINS OF NEURON MEMBRANE

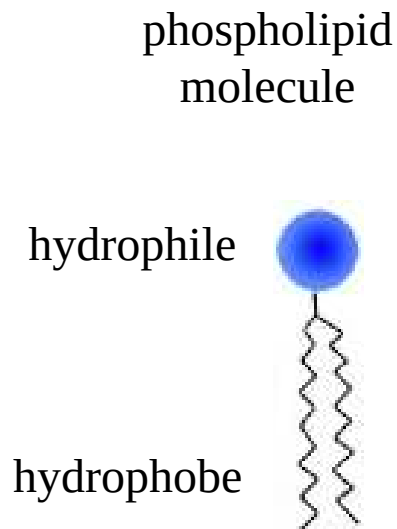
Voltage Gated Channel



Ligand Gated Channel

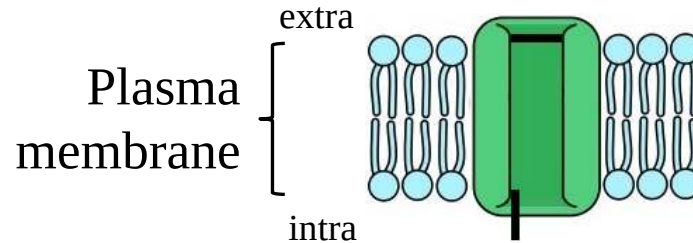


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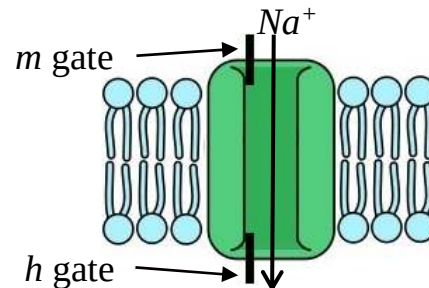
VOLTAGE-GATED Na^+ CHANNEL

REST



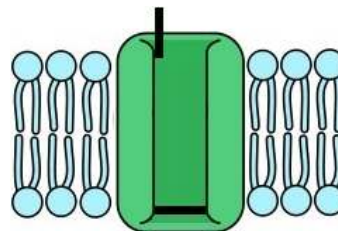
At rest
($V_m = -75 \text{ mV}$)

OPEN



Immediately after
depolarization ($V_m = -50 \text{ mV}$)

INACTIVE



5 msec after
depolarization ($V_m = -50 \text{ mV}$)

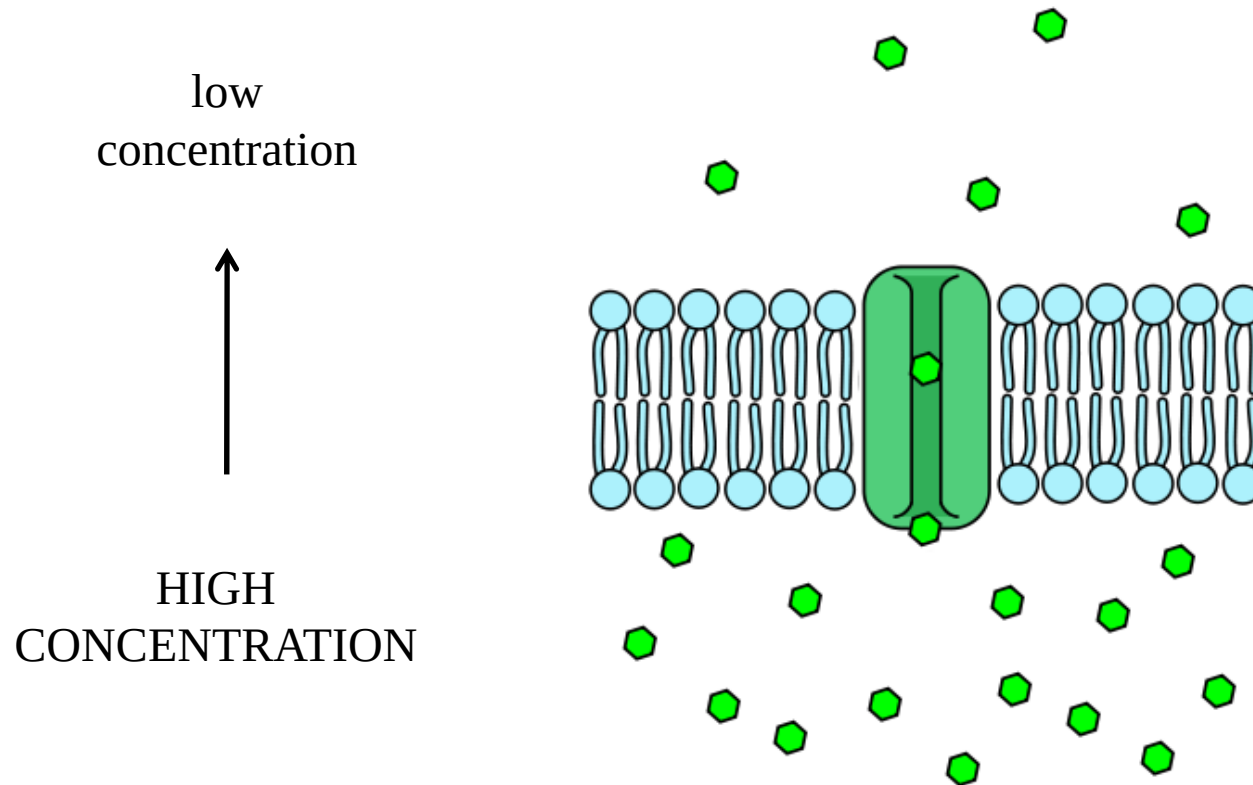
VOLTAGE-GATED Na^+ CHANNEL

At resting potential, the m gate of the channel is closed, therefore Na^+ ions are not able to flow through the membrane.

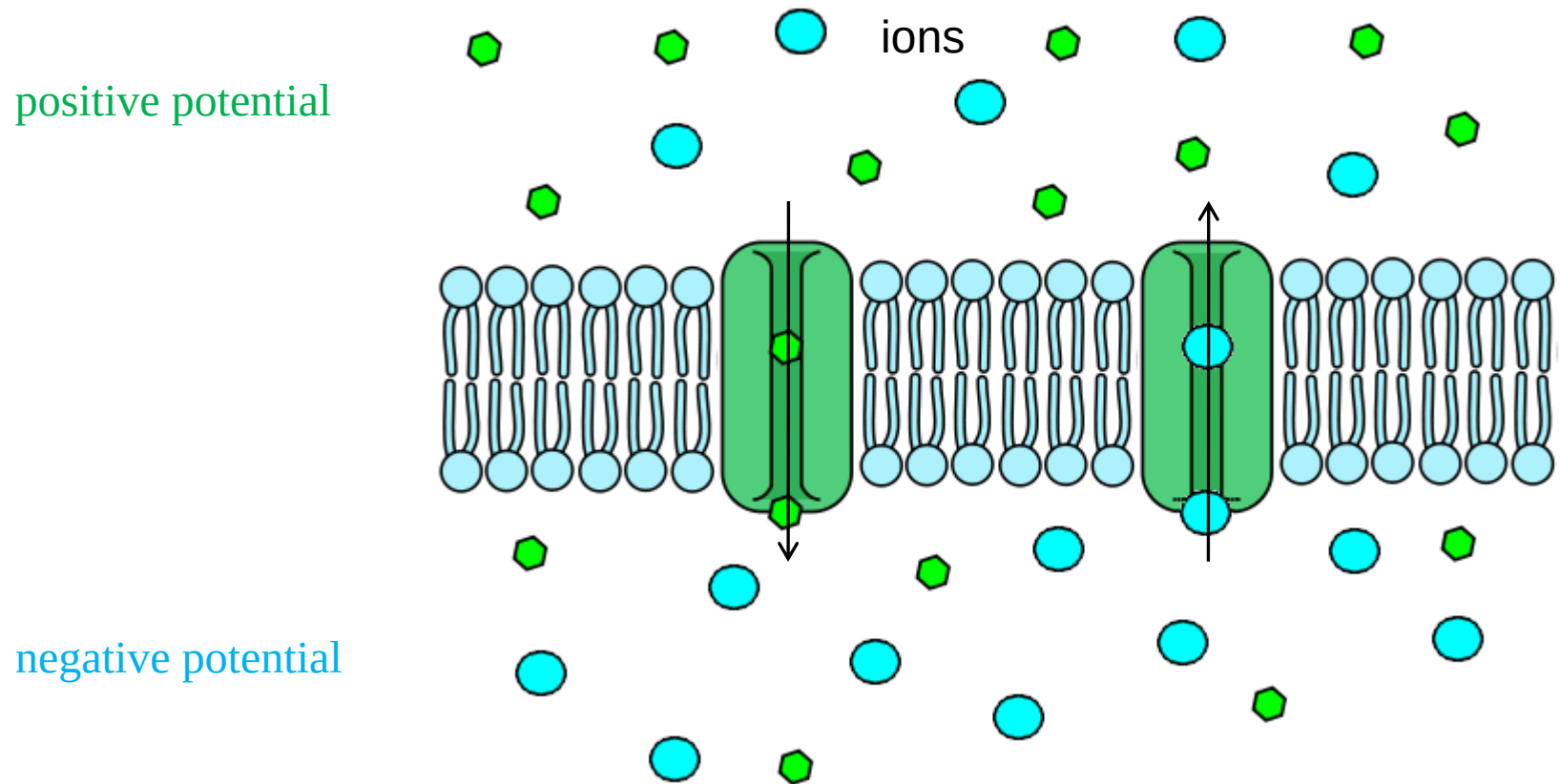
When the membrane is depolarized (the potential difference between the two sides of the membrane is smaller), both gates of the voltage-gated Na^+ channel open, allowing the Na^+ ions to flow into the cell, further depolarizing the membrane.

After the depolarization the h gate of the channel closes for a few milliseconds, stopping the inward flow of Na^+ .

DIFFUSION BETWEEN SPACES WITH DIFFERENT CONCENTRATION



ION MOVEMENT IN ELECTRIC SPACE



ION MOVEMENT

The movement of ions through the membrane depends on

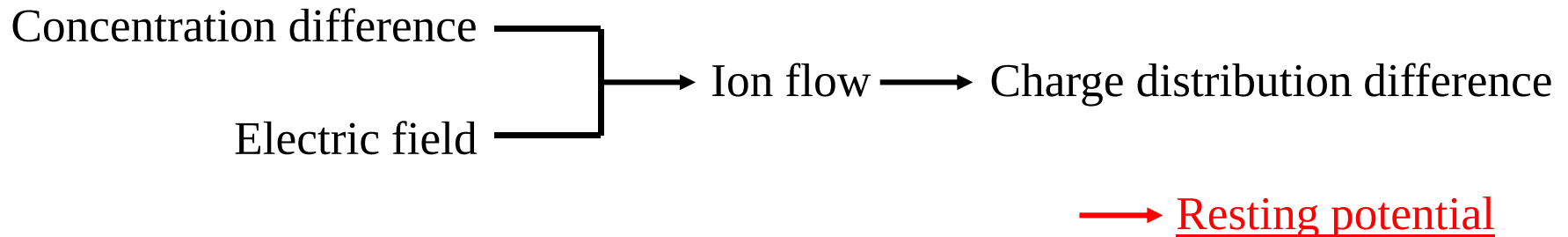
- the ion concentration gradient,
- electric charges.

If the concentration of a certain molecule is higher in one compartment than the other, they will diffuse to the compartment with lower concentration (diffusion force).

If the electric field is positive in one compartment, negative ions will tend to move there, while positive ions will move away and vice versa (electrostatic force).

Furthermore, ion movement is determined also by the type of open channels. Some channels are selective for ions (e.g. only cations, or only K⁺ ions).

ION MOVEMENT

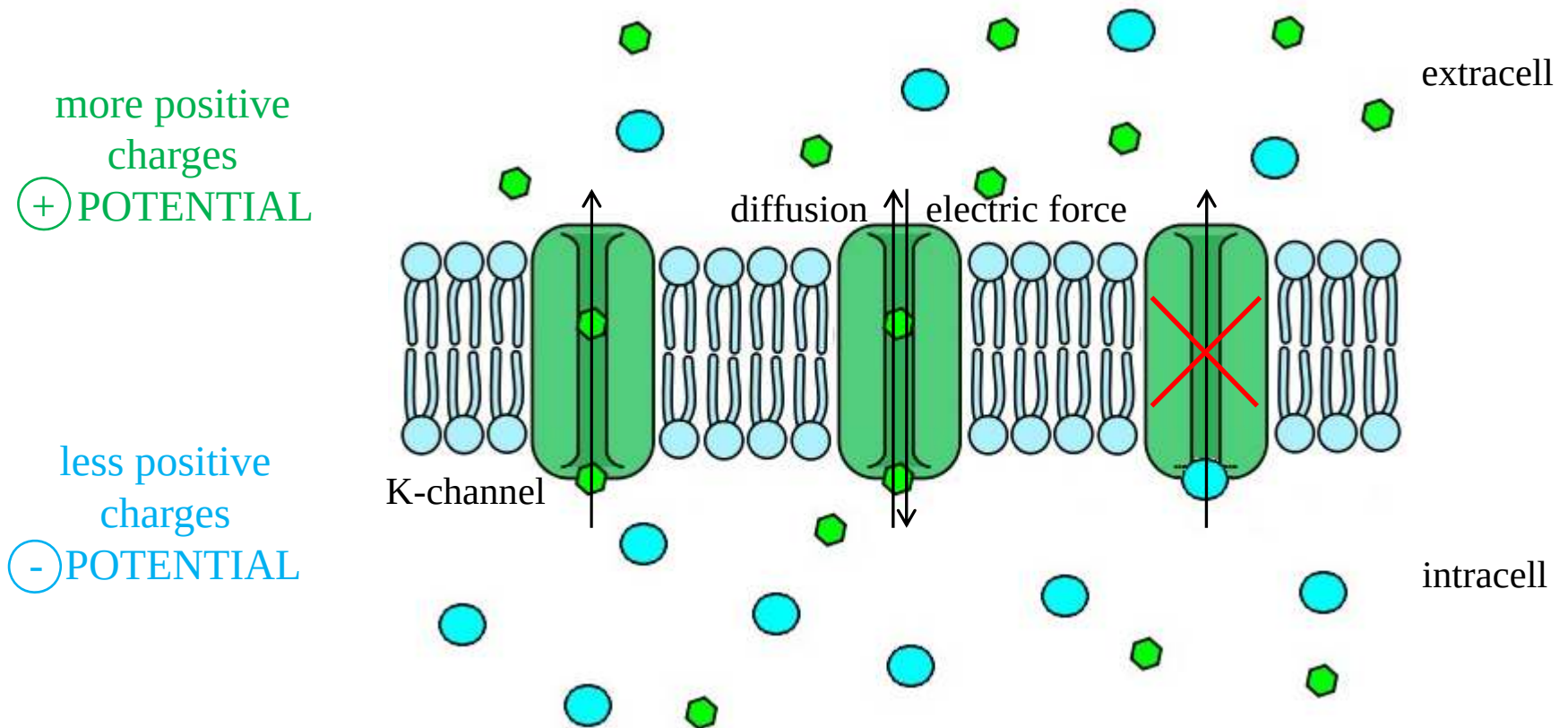


In a living cell $[K^+]_{\text{intracell}} > [K^+]_{\text{extracell}}$ and $[Na^+]_i < [Na^+]_e$

In a living cell at rest K^+ flows through the membrane much more easily than any other ions. If $p_K=1$ then $p_{Na}=0.1$.

Very few ions are transported, only small changes in concentration take place.

ION MOVEMENT THROUGH SELECTIVE CHANNEL



ION MOVEMENT

Electric field

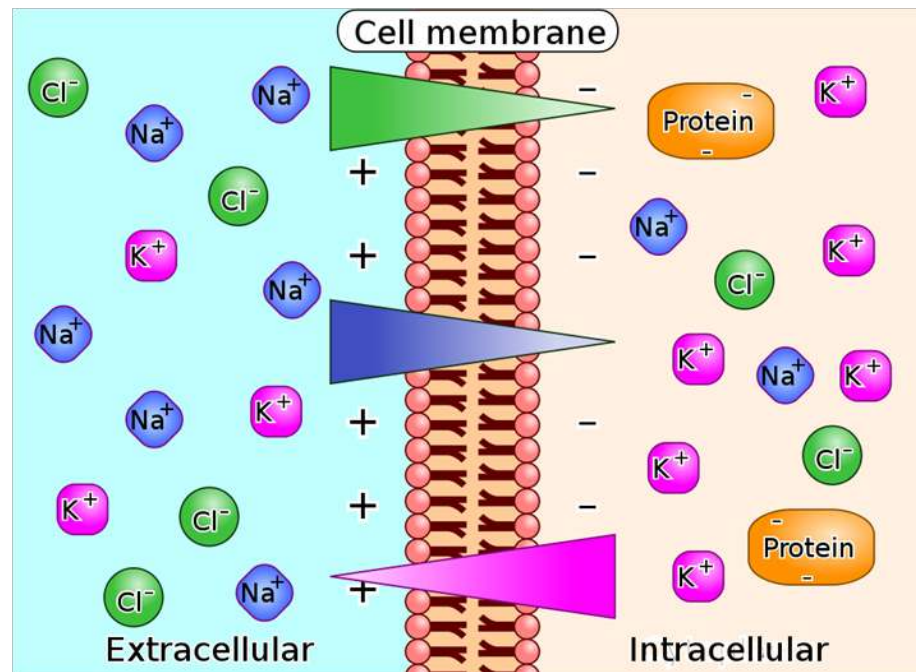
$$j_{drift,p} = \frac{Z_p}{[Z_p]} \cdot C_p \cdot v_p = \frac{Z_p}{[Z_p]} \cdot C_p \cdot (u_p \cdot E)$$

Diffusion

$$j_{diffusion} = -D \cdot \nabla C$$

drift flux
valence
concentration
velocity
mobility

j_{drift}
 Z_p
 C_p
 v_p
 u_p



diffusion flux
diffusivity
concentration

$j_{diffusion}$
 D
 C

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Ion diffusion: $j_{diffusion} = -D \cdot \nabla C$

Electric field: $j_{drift,p} = \frac{Z_p}{|Z_p|} \cdot C_p \cdot v_p = \frac{Z_p}{|Z_p|} \cdot C_p \cdot (u_p \cdot E)$

$$j_p = j_{diffusion,p} + j_{drift,p} = -D_p \cdot \left(\nabla C_p + \frac{Z_p \cdot C_p \cdot F}{R \cdot T} \cdot \nabla V \right)$$

$$J_p = j_p \cdot (F \cdot Z_p) \quad E = -\nabla V$$

$$J_p = -D_p \cdot (F \cdot Z_p) \cdot \left(\nabla C_p + \frac{Z_p \cdot C_p \cdot F}{R \cdot T} \cdot \nabla V \right)$$

$$D_p = \frac{u_p \cdot R \cdot T}{|Z_p| \cdot F}$$

No net current flow in equilibrium:

$$J_p = -D_p \cdot F \cdot Z_p \cdot \left(\nabla C_p + \frac{Z_p \cdot C_p \cdot F}{R \cdot T} \cdot \nabla V \right) = 0$$

R = gas constant = 8.314 J/K mole at 27 °C

F = Farady = 96,487 coulombs/mole

Nernst equation

$$V_m = (V_i - V_e) = \left(\frac{R \cdot T}{Z_p \cdot F} \right) \cdot \ln \left(\frac{[C_p]_e}{[C_p]_i} \right)$$

NERNST EQUILIBRIUM

- ion selective membrane
- ion concentration difference
- mobile + ion (K, intracell)
- non mobile – ion (A, intracell)

V_k = equilibrium potential

F = Faraday constant [9.649×10^4 C/mol]

T = absolute temperature [K]

R = gas constant [8.314 J/(mol·K)]

z_k = valence

$c_{i,k}$ = intracell concentration

$c_{o,k}$ = extracell concentration

Nernst equation

$$V_k = -\frac{RT}{z_k F} \ln \frac{c_{i,k}}{c_{e,k}}$$

$$T = 273 + 37$$

$$z = 1$$

$$V_k = -61 \cdot \log_{10} \frac{c_{i,k}}{c_{e,k}} \text{ [mV]}$$

$$V_m = V_k = V_i - V_e$$

V_m : membrane potential

$$c_{i,K} = 120 \text{ mmol/l}$$

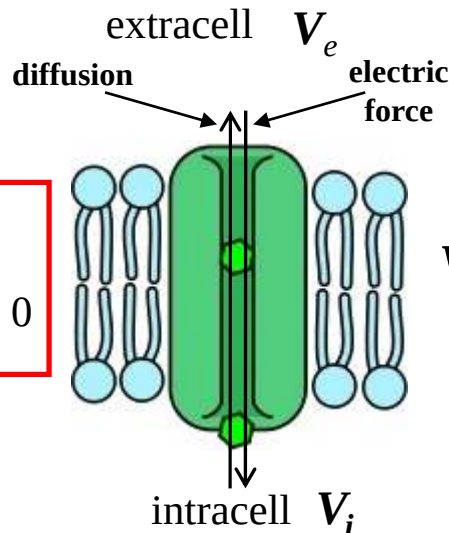
$$c_{e,K} = 5 \text{ mmol/l}$$

$$V_k = 61 \log_{10}(24)$$

$$V_K = -84.19 \text{ mV}$$

Equilibrium

$$I_{\text{DIFF}} + I_E = I_{\text{net}} = 0$$



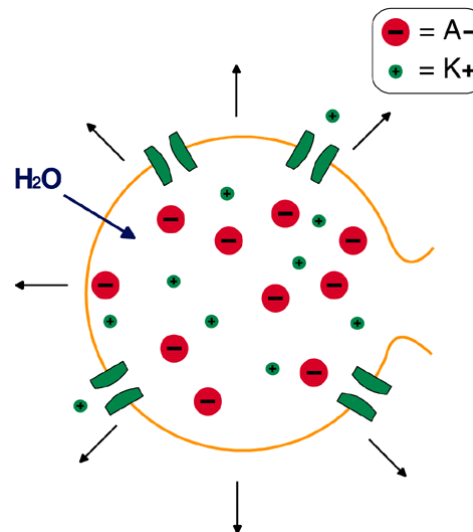
NERNST EQUILIBRIUM

For an excitable cell, a dielectric membrane and ion concentration difference on its two sides are essential.

Resting membrane potential is the membrane potential (the potential difference between the two sides of the membrane) when there is no net ion movement. It is an equilibrium state when the sum of diffusion and electrostatic forces is zero. It also means there is no net current flow through the membrane.

The Nernst equation gives the equilibrium potential of an ion, given its intra- and extracellular concentrations. This is the potential when there is no net movement of that ion. This value is proportional to the logarithm of the quotient of concentrations.

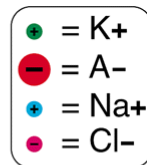
OSMOTIC CATASTROPHE



Due to high concentration of ions inside the cell, water would diffuse into the cell until it bursts.

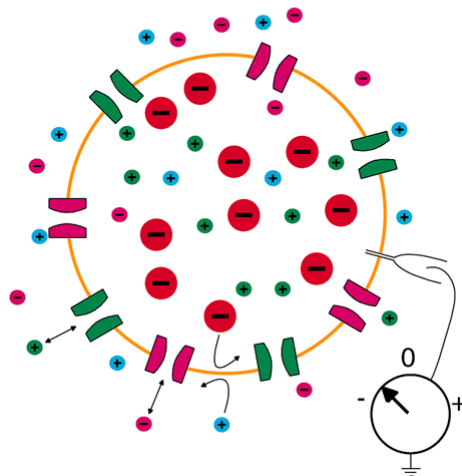
COMPENSATE FOR THE DIFFUSION OF WATER

NaCl in the extracell space!



Equilibrium

$$I_{\text{DIFF}} + I_E = I_{\text{net}} = 0$$



- ion selective membrane
- ion concentration difference
- mobile + ion (K, intracell)
- non mobile – ion (A, intracell)
- mobile – ion (Cl, extracell)
- non mobile + ion (Na, extracell)
- compensated for water diffusion: $c_i = c_e$
- $c_{i,K} = c_{e,Cl}$ $c_{e,K} = c_{i,Cl}$

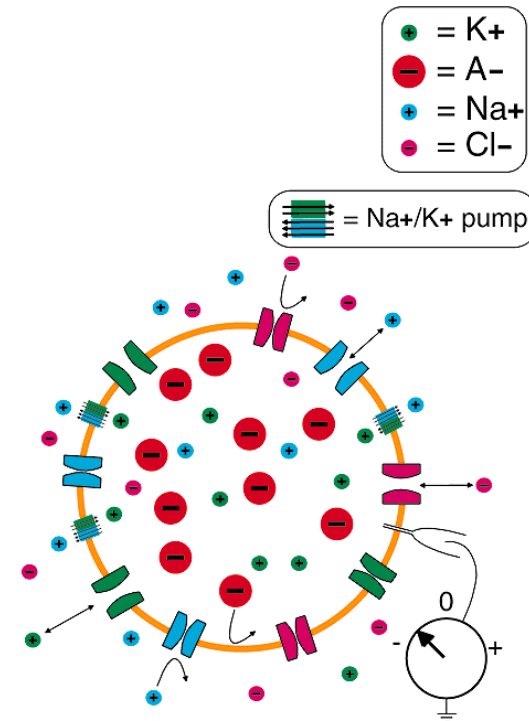
$$V_D = V_{Cl} = V_K = V_m = V_i - V_e$$

$$V_D = -61 \cdot \log_{10} \frac{c_{i,K} + c_{e,Cl}}{c_{e,K} + c_{i,Cl}} [\text{mV}]$$

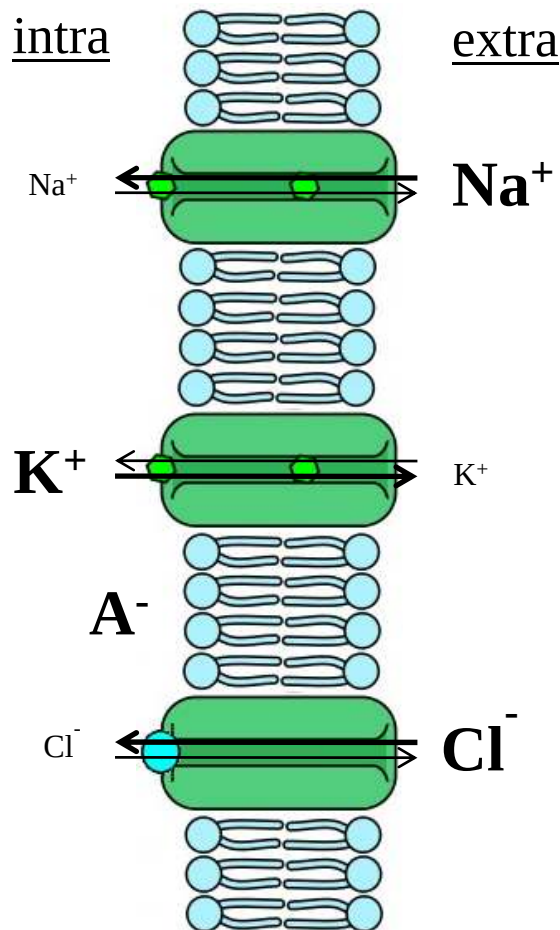
$$V_D = V_K = V_{Cl} = V_m = -84.19 \text{ mV}$$

REALISTIC CELL MODEL

- ion selective membrane
- ion concentration difference
- mobile + ion (K, intracell)
- non mobile – ion (A, intracell)
- mobile – ion (Cl, extracell)
- non mobile + ion (Na, extracell)
- compensated for water diffusion
- different permeability of ion channels
- active transport for maintaining ion gradient (Na/K pump)
- no equilibrium on ion channels (leak)



RESTING POTENTIAL



ion	intra [mmol/l]	extra [mmol/l]	V_k
Na^+	15	150	+61 mV
K^+	120	5	-84.19 mV
Cl^-	7.5	125	-74.53 mV

ion	permeability, P [cm/sec]
Na^+	0.05×10^{-7}
K^+	1×10^{-7}
Cl^-	0.1×10^{-7}

$V_r = -61.15 \text{ mV}$

$$P_K > P_{Cl} > P_{Na}$$

Goldman-Hodgkin-Katz equation

$$V_r = -61 \cdot \log_{10} \frac{P_K \cdot c_{i,K} + P_{Na} \cdot c_{i,Na} + P_{Cl} \cdot c_{e,Cl}}{P_K \cdot c_{e,K} + P_{Na} \cdot c_{e,Na} + P_{Cl} \cdot c_{i,Cl}} [\text{mV}]$$

Depolarization: $V_m > V_r$

Hyperpolarization: $V_m < V_r$

CHANGES IN MEMBRANE POTENTIAL

Depolarization: $V_m > V_r$

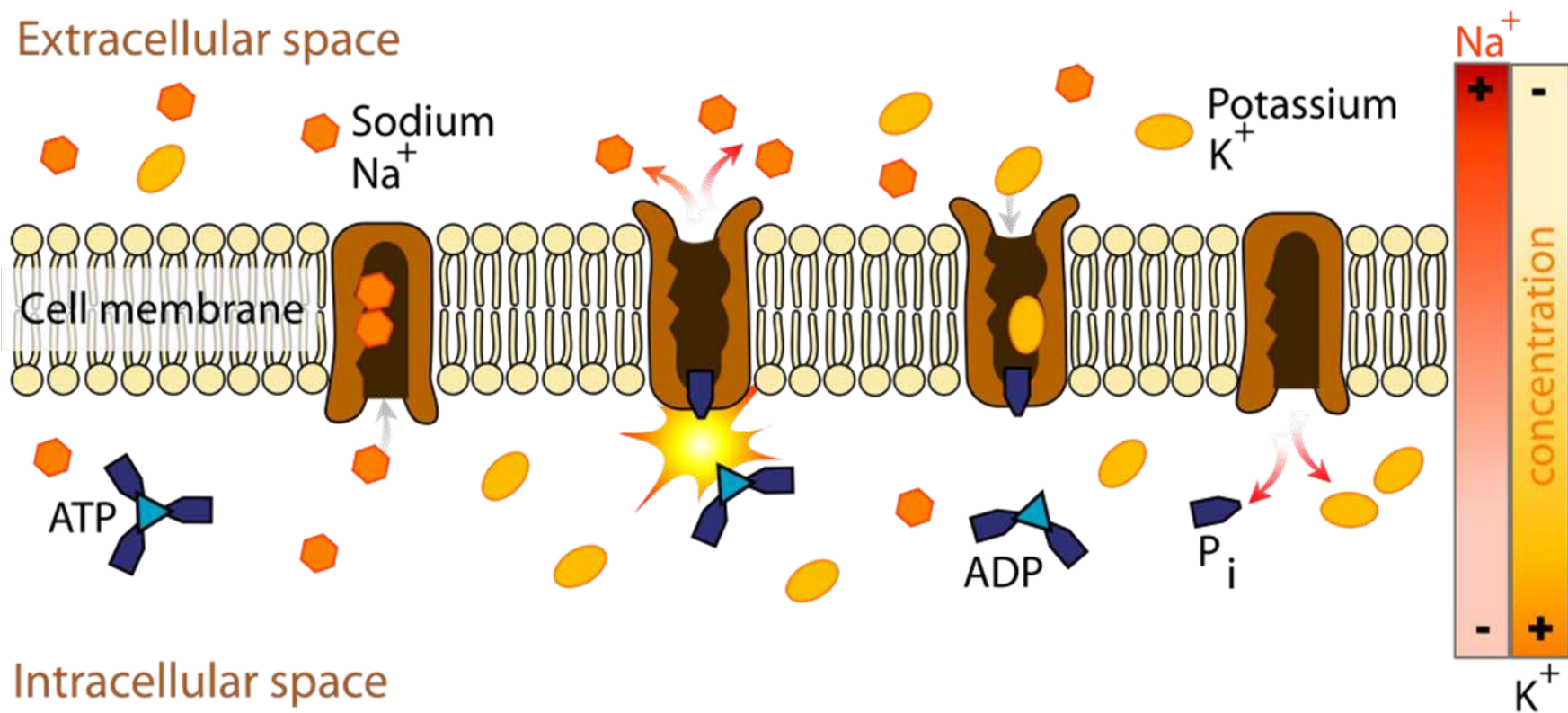
Hyperpolarization: $V_m < V_r$

P constant	Extraracell concentration	V_m	Extraracell concentration	V_m
Na^+	↑	D	↓	H
K^+	↑	D	↓	H
Cl^-	↑	H	↓	D

Concentration constant	P	V_m	P	V_m
Na^+	↑	D	↓	H
K^+	↑	H	↓	D
Cl^-	↑	H/D	↓	D/H

Channels wide open push the membrane potential to the equilibrium potential of given ion.

SODIUM-POTASSIUM PUMP



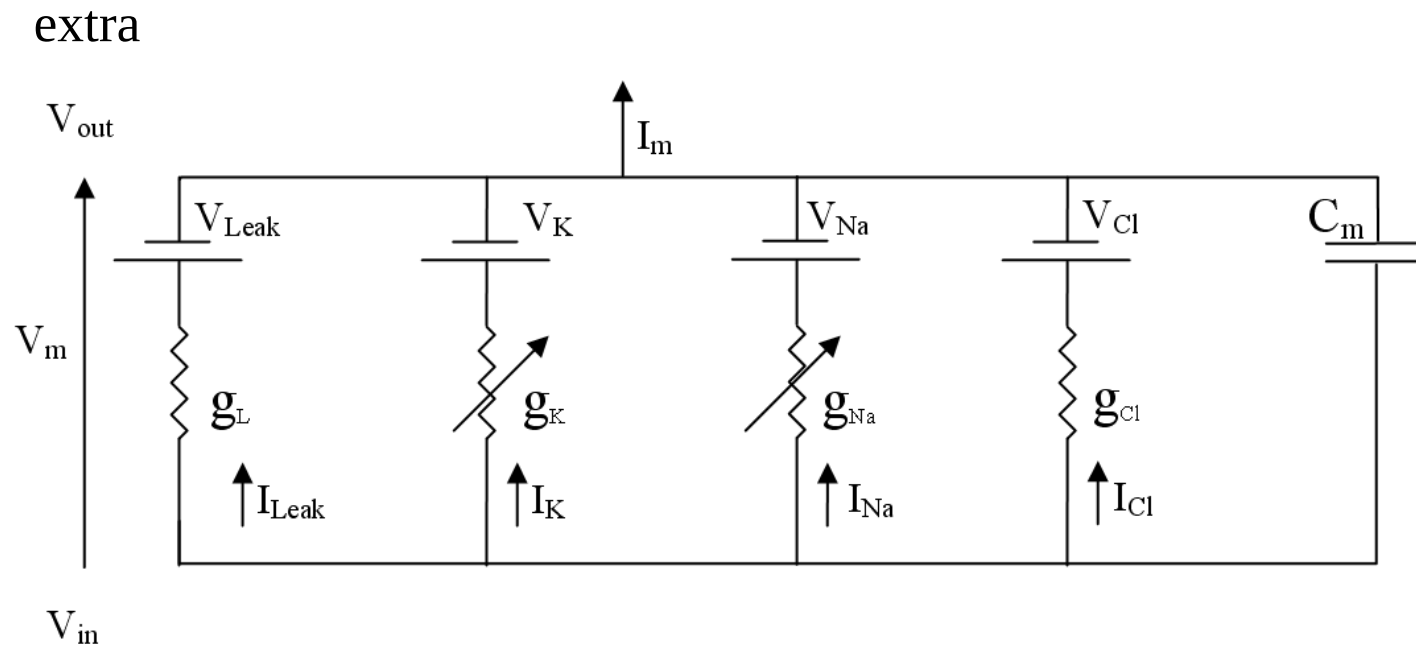
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SODIUM-POTASSIUM PUMP

In order to maintain the physiological ion concentrations, ions transported through the membrane have to be transported back. This happens against their concentration gradient, thus requires energy.

This task is executed by ion pumps and ion transporters. The sodium-potassium pump takes three sodium ions back to the extracellular space and two potassium ions to the intracellular space. It uses ATP dephosphorylation to acquire the energy needed for the transport.

MEMBRANE EQUIVALENT CIRCUIT



intra

$$R=U/I$$

$$G=1/R \text{ (conductance)}$$

(commons.wikimedia.org)

REFERENCES

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SUMMARY

- ionselective (semipermeable) membrane
- membrane permeable for water, not permeable for ions
- condition for excitation: ion concentration difference between the two sides of the membrane
- voltage on membrane depends on diffusion and electrostatic forces
- in Nernst equilibrium the voltage is proportional to the logarithm of the quotient of concentrations
- in Nernst equilibrium there is no net current flow on ion channels
- resting potential is determined by ion concentration differences and ion channel permeabilities
- the most important mobile ions are Na, K, and Cl
- intracellularly many negatively charged proteins and K, extracellularly many Na and Cl ions
- $P_K > P_{Cl} > P_{Na}$

SUMMARY

- ions in dynamic balance, no real equilibrium, leaky channels
- leak compensated by energy demanding pumps/transporters (Na-K pump)
- Goldman equation gives good approximation for resting potential
- depolarization $V_m > V_r$, hyperpolarization $V_m < V_r$
- increasing permeability of ion channel pushes resting potential towards equilibrium (Nernst) potential of given ion
- increasing P_{Na} depolarizes (inward Na flow),
increasing P_K hyperpolarizes (outward K flow)
- increasing P_{Cl} may either depolarize or hyperpolarize, depending on equilibrium potential of Cl

REVIEW QUESTIONS

- What is the structure of a neuron membrane?
- What types of ion channels do you know?
- What forces drive the ions through the membrane?
- What is the Nernst equilibrium?
- What does the Goldman-Hodgkin-Katz equation tell?