Resting potential, action potential and electrotonic potentials

- Ionic mechanisms -

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2024

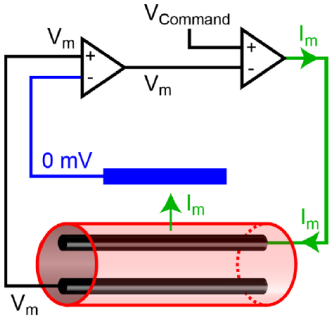
By Péter Sántha
modified by Levente Gellért

Learning objectives: 4-6

Transmembrane potential

Resting membrane potential (E_0):

Transmembrane electric potential difference in the cells measured under resting conditions (in absence of any influence which might alter the membrane potential)
Cell specific: **-90 - -50 mV**
The value is determined by ionic conductances and transport mechanisms, and the Donnan potential



giant axon of the squid

Biological significance:
•Signalisation and signal propagation
•Driving force for transport processes
•Regulation of the cell volume

In majority of the cells its value is stable
maintenance of constant E_0 requires metabolic energy (up to 70% of the total ATP consumption of the cells!!)
Cells with unstable E_0 might act as **pacemaker cells** (generation of rhythmic activity-APs; e.g.: cells of the **nodal tissues of the heart**; interstitial cells of Cajal – GI tract)

Asymmetric distribution of ions in extra- and intracellular fluid compartments:

	ECF (mmol/L) (interstitial fluid)	ICF (mmol/L) (cytosol)
Na^+	150	15
Cl^-	100	5
K^+	4.5	150
Ca^{2+}	1.8	0.0001
H^+	0.00004 (pH=7.4)	0.0002 (pH=6.7)

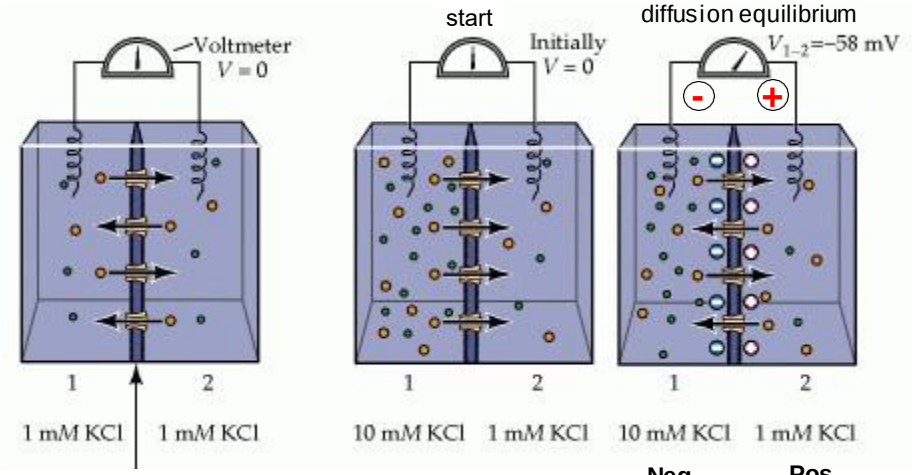
Plasma membrane

Electroneutrality: phosphate sulphate, lactate

Electroneutrality: protein anions

Attraction or repulsion of charged particle in electric field

Development of diffusion potential - charge separation - the Nernst potential
Equilibrium: there is no net diffusion of permeable ion(s)
charge separation: Cations and anions accumulate close to the inner and outer surfaces of the membrane – electrostatic field develops
Nernst potential – the resulted potential is proportional with the concentration difference



K^+ permeable membrane

charge separation – electrostatic field

The Nernst equation: determines the equilibrium potential of a given ion having different $[ion]_o$ (ECF) and $[ion]_I$ (ICF) concentrations:

$$E_{ion} = \frac{RT}{ZF} \times ln \frac{[ion]_o}{[ion]_i}$$

Z = valence of the ion
R = gas constant
F = Faraday constant
T = temperature

$$E_{K^+} = 61.5mv \times \log_{10} \frac{[K^+]_o}{[K^+]_i}$$

T=37 °C

Calculated equilibrium potentials for different ions (see data before):

- $E_{Na} = 60$ mV log 150/15 = + 60 mV
- $E_{Ca} = 60/2$ mV log 1.8/0.0001 = + 130 mV
- $E_K = 60$ mV log 4.5/150 = - 90 mV
- $E_{Cl} = 60/-1$ mV log 100/5 = -80 mV

Different ions have different equilibrium potentials
These values also differ from the resting membrane potential

To explain the development of a **stable resting potential** we have to consider:
+ the intensity and direction of **passive ion fluxes** (most importantly Na^+; K^+; Cl^-)
+ **active transport processes** (electrogenic ion pumps!)
+ (**Donnan effect – immobile charged macromolecules in the ICF**)

Prerequisite for a stable membrane potential: the **algebraic sum** of the ion fluxes should be **zero** – there should be a dynamic balance between the inward and outward ion currents!

Ohm`s law: $R = U / I \rightarrow I = U / R$ *and* $I = U \times g$ *(g=conductance)*

Electrostatic driving force (E_i) =??

It is the difference between the actual E_m and the Nernst potential of the ion:
$\rightarrow E_i = E_{Nernst} - E_m$;

Calculation of the net K^+ current: $I_{K+}=(E_{K+} - E_m) \times gK^+$

$$\Sigma I_{net} = 0 = I_{K+} + I_{Na+} + I_{Cl-} = g_{K+} \times Ei_{K+} + g_{Na+} \times Ei_{Na+} + g_{Cl-} \times Ei_{Cl-}$$

Goldmann-Hodgkin-Katz (GHK) equation:

Determines the membrane potential at which a diffusion equilibrium develops at the given ion concentrations and membrane conductance (permeability) values

$$E_m = \frac{RT}{F} \cdot \ln \frac{P_K[K^+]_a + P_{Na}[Na^+]_a + P_{Cl}[Cl^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_a}.$$

Empirically the resting permeability (conductance) values of the plasma membrane are:

$P_K : P_{Na} : P_{Cl} = 1 : 0{,}04 : 0{,}45$

High resting **K^+** permeability: resting potential is close to the equilibrium pot. of K^+ !

Any change in these parameters results in a change of the membrane potential!!

E_m becomes more negative: **Hyperpolarization**
E_m becomes more positive: **Depolarization**

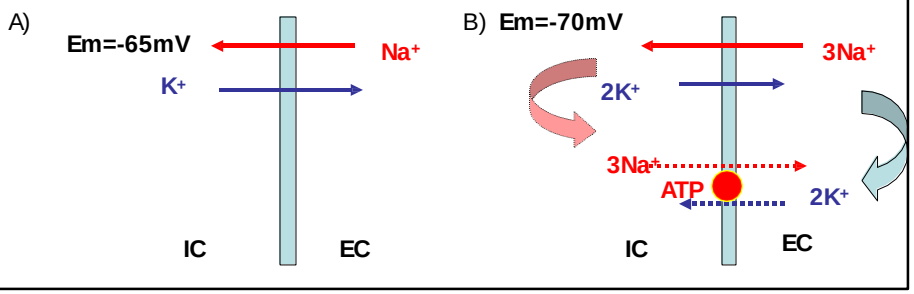
Changes in the ion concentrations:
$[K^+]$ in ECF increases (hyperkalemia): **depolarization** – (arrhythmias, cardiac arrest)
$[K^+]$ in ECF decreases (hypokalemia): **hyperpolarization** – (arrhythmias, PNS failures)
These changes can cause emergency situations!!

Changes in the conductances (activity of the ion channels) cause:
phasic (rapid) changes: action potentials
tonic (slow) changes: post synaptic potentials, sensory (generator) potentials, etc.

Further problem: **continuous inward and outward diffusions of ions** (fig. A) would abolish the concentration gradients → finally the E_m would be stabilized at 0 mV

In the living cells an **electrogenic active transport system** maintains a stable negative resting potential (fig. B)
the role of **Na^+ -K^+ ATPase (see Membrane 1 lecture!)**

Ratio of the antiport mechanism is 3 Na^+ outward pro 2 K^+ inward (net 1+ outward/cycl.)
This shifts the calculated Em (GHK equation) with cc. **-5 mV** to the negative direction
-**hyperpolarising pump potential**
Consequnce:
Inhibition of the Na-K ATPases (e.g.: ouabain, hypoxia) **depolarizes** the membrane.
Reduction in the E_m causes Cl^- (and **Na^+) influx and swelling** of the cells (e.g.: in the CNS brain edema develops) → Na^+ -K^+ ATPase regulates the **cell volume**!!!



Importance of the membrane capacitance

The electric charges (free ions, bound charges), which maintain the transmembrane potential are stored close to the inner and outer surfaces of the plasma membrane: Plasma membrane acts as a **capacitor** (lipid bilayer acts as an insulator).

Placing different amounts of charge on the two sides of the plasma membrane, **membrane potentials** are generated, which depend upon the charge and the capacitance of the lipid bilayer

U=Q/C (charge/capacitance)

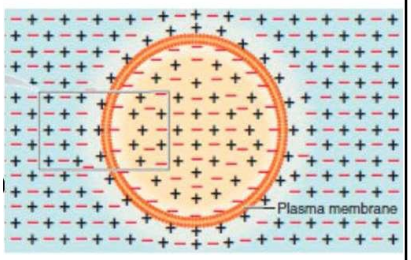
Example:

capacitance per unit area is around 1 μF/ cm^2

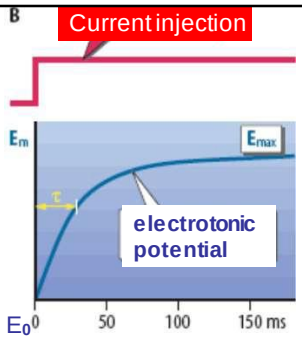
10 μm - 10 pF

100 mV - 1 pC - $6 \cdot 10^6$ potassium ions

Total:~10^{11} K+ inside an individual cell!!

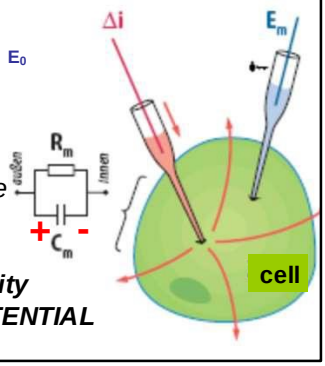


***Tonic changes* of the membrane potential: electrotone - electrotonic potentials**



Stimulation with an intracellular microelectrode
Inward current of positive charges is driven by an external current source

1.partial discharge of the capacitor (quick depolarization)
2. increase of the compensatory passive efflux of cations (slow depolarization and steady state)



inward current (+ charges) – **depolarization**
outward current (+ charges) – **hyperpolarization**

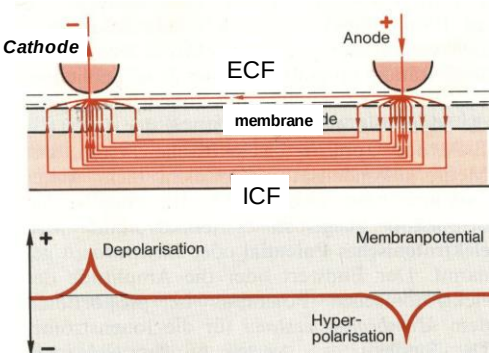
The current injection-induced change in the membrane potential is called as ***electrotonic potential (electrotone)***
ΔE_m (E_{max}) is proportional with the stimulus intensity *and the* ***membrane resistance (R_m) – GRADED POTENTIAL***

Extracellular stimulation:

Cathode
- *depolarization of the membrane (cathelectrotone)*

Anode
- *hyperpolarization of the membrane (anelectrotone)*

The propagation of the electrotonic potentials is limited in space- „propagation with decrement"
LOCAL POTENTIALS



Applications in the medical praxis*:*

Ventricular tachycardia (emergency!!) – ***electro-cardioversion*** *and* ***defibrillation***
Pacemaker therapy *(heart, diaphragm, CNS)*
Electroconvulsive Therapy (Psychotic patients)
Endocochlear implants („artificial inner ear")
TENS: Transdermal Electric Nerve Stimulation (pain therapy)

Types of intrinsic electrotonic potentials (EPs)
(also called as **graded** - or **local** potentials)

Intrinsic= EPs without external electric stimulation

Postsynaptic Potentials (PSPs)
activation of **ligand gated ion channels** – ionotrop receptors
Indirect regulation of ion channels by transmitters – **metabotrop receptors** (signal transduction – second messengers)

Receptor (generator) potentials
sensory neurons and sensory epithelial cells - ion channels operated by sensory signals (mechano-, thermo –, and chemo-sensitive channels, etc.)
„sensory transduction" process

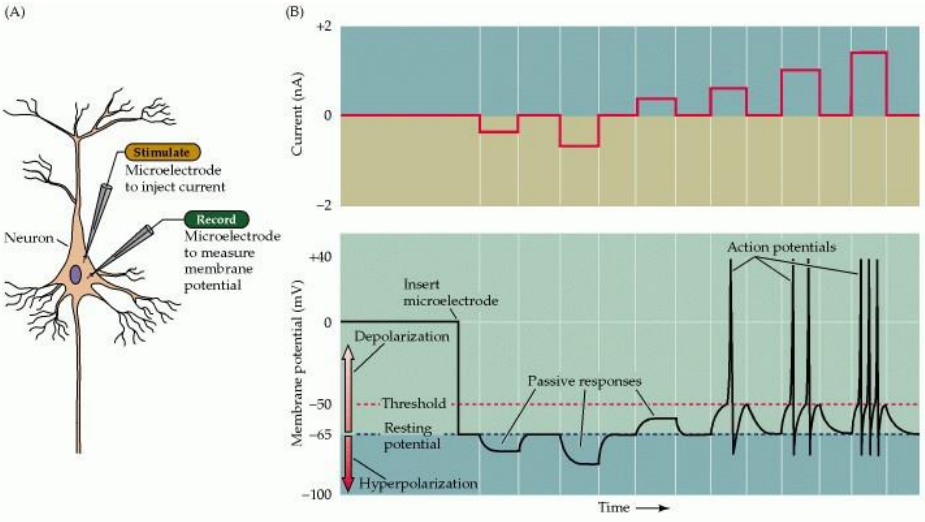
Propagation of the action potentials
Passive currents evoked by the ion fluxes through voltage gated channels

Pacemaker potentials
spontaneous depolarization of the membrane evoked by operation of special ion channels

Major characteristics of the electrotonic (graded/local) membran potentials

Activation threshold	no threshold, „obligate"
Sign of the potential change	either de- or hyperpolarizing (stimulus dependent)
Amplitude	graded (stimulus dependent) – „analog" signal
Propagation	with decrement – local change
Rephracterity	no rephractory period
Summation	temporal and spatial superposition of EPs of different origin
Mechanism	„passive" membrane currents; opening/closure of ion channels, electrical stimulation
Biological function	Signal conduction and processing (PSPs) Sensory transduction Pacemaker activity

Recordings of passive and active electrical signals in a nerve cell



Neuroscience Purves, Dale; Augustine, George J.; Fitzpatrick

Why do we need action potential?

(That is, a regenerative digital signal)

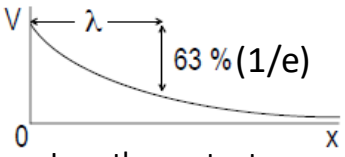
Propagation of electrotonic potentials along elongated membrane structur

(leaky electrical cables with capacitance)

The amplitude of the EP shows decay with the distance: **decrement**



Strength of depolarising current decreases with the distance (inhomogenous current distribution)

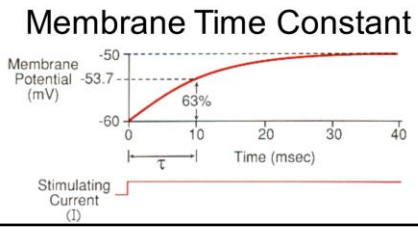


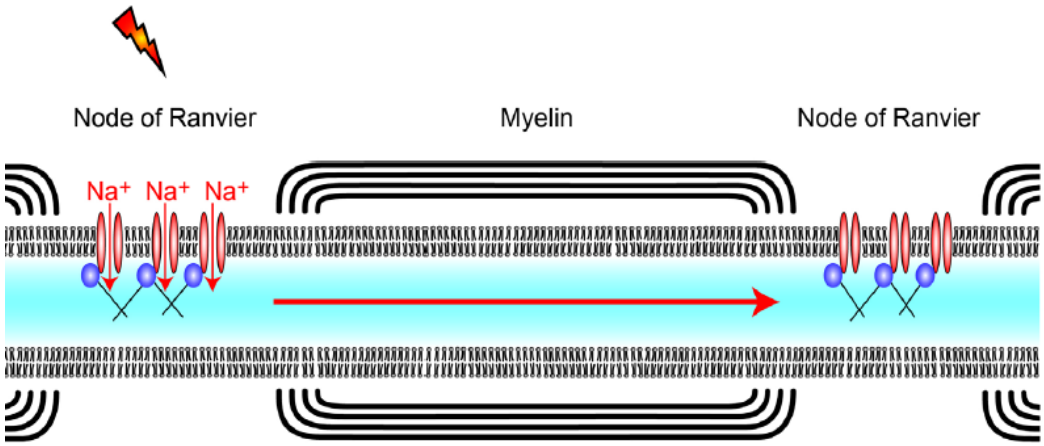
Length constant

Local circuits model (cable-theory)
R_m – membrane resistance
R_a – axon (axial) resistance

Length constant: distance where
$E_m = E_{max}$ x 0.37% (100%-63%)

- directly proportional to R_m
- inversely proportional to R_a

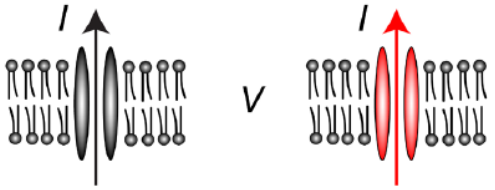




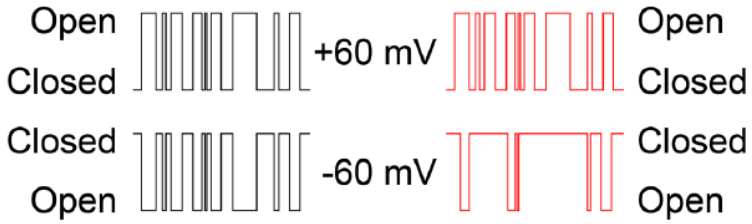
decreases the axonal capacitance by a factor of around 50

increases the axonal membrane resistance by a factor of 5,000

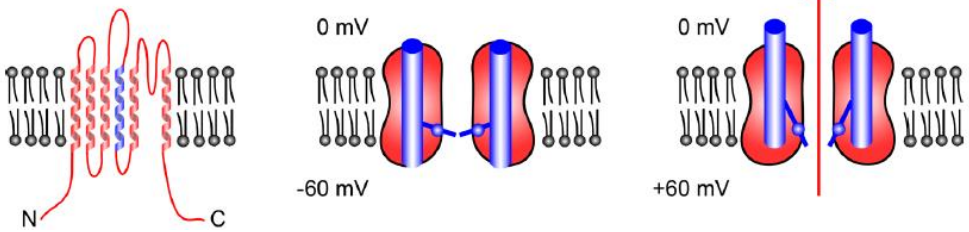
linear ionic conductance

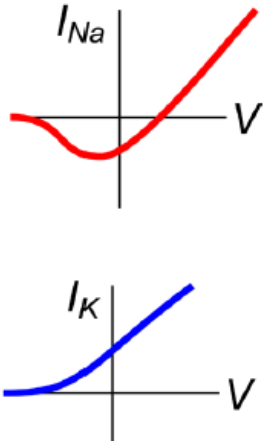


non-linear ionic conductance

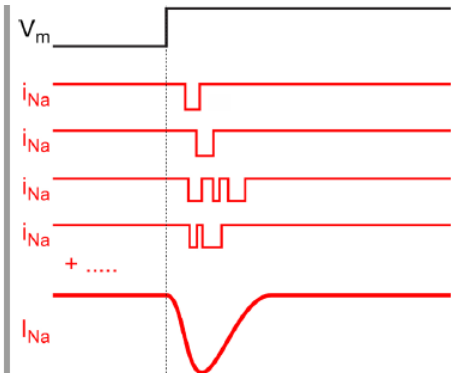


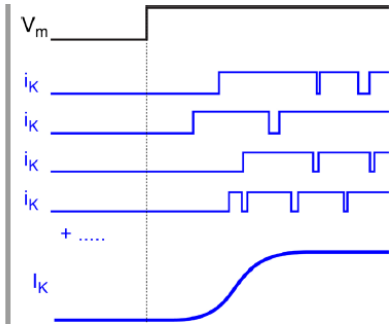
non-Linear ionic conductances: voltage-gated sodium and potassium channels

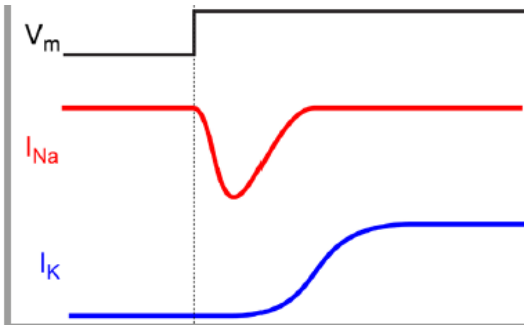


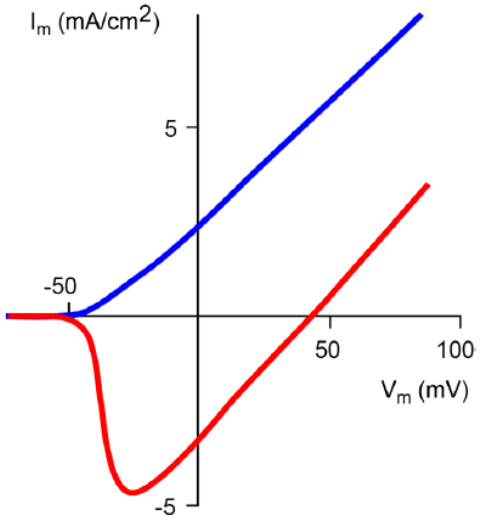


voltage-gated sodium and potassium channel kinetics









Conduction velocities of nerve fibres





Unmyelinated (C-fibres)
1 m/s -- 3,6 km/h

Myelinated (A alpha)
fibres 100 m/s – 360 km/h

Conduction velocity of the axons

Conduction velocity (0.5 – 100 m/s) depends on:

- Strength of depolarizing inward currents (activity of Na_V^+ channels – see local anaesthetics: they inhibit a Nav channels and the propagation of AP in nerves)
- Physical („passive") parameters of the axon and the membrane:

the conduction velocity is –

directly proportional to:
transmembrane resistance

inversely proportional to:
axonal length resistance –(determined by axonal diameter - squid axon Ø is ~1mm!!)
membrane capacitance - (determined by thickness of the membrane/myelin)

Vertebrates: **myelinisation** of axons – *reduces* the membrane capacitance and *increases* the membrane resistance!

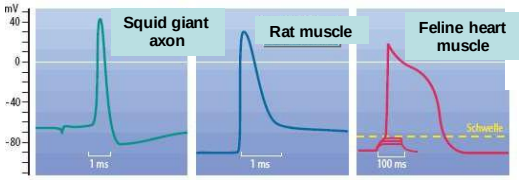
Action potential

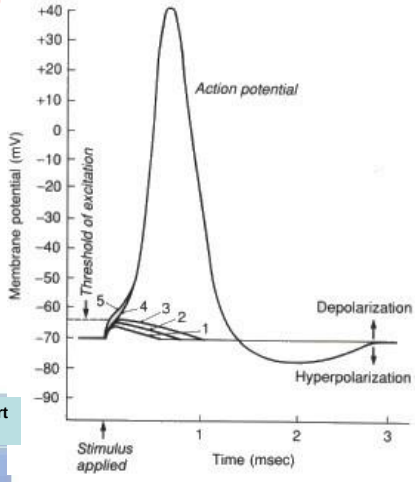
Excitable cells – neurons and muscle cells **Action potential:** a transient, stereotype, depolarising, self-regenerating change in the membrane potential evoked by supra-threshold stimulus (depolarisation). + Voltage-gated ionchan.

Phenomenology:

Rapid depolarization of the membrane induced by external or internal (e.g.: pacemaker cells) stimuli

Stereotype: shape, length and amplitude are constant and independent of the parameters of the stimulation (**all-or –none principle**)

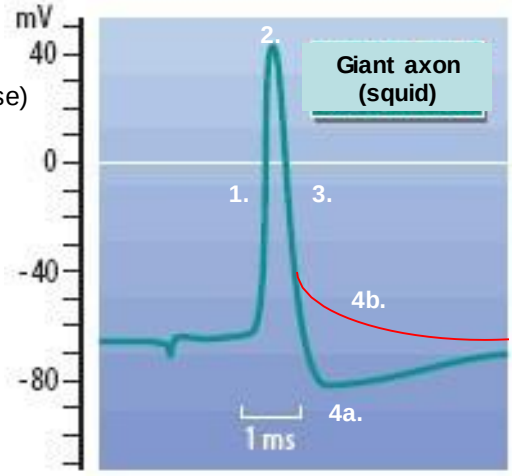




Phases of the action potential

1. rapid depolarization (rising phase)
2. peak (overshoot)
3. repolarization (falling phase)

Afterpotentials (cell specific):
4a. hyperpolarizing
4b. depolarizing

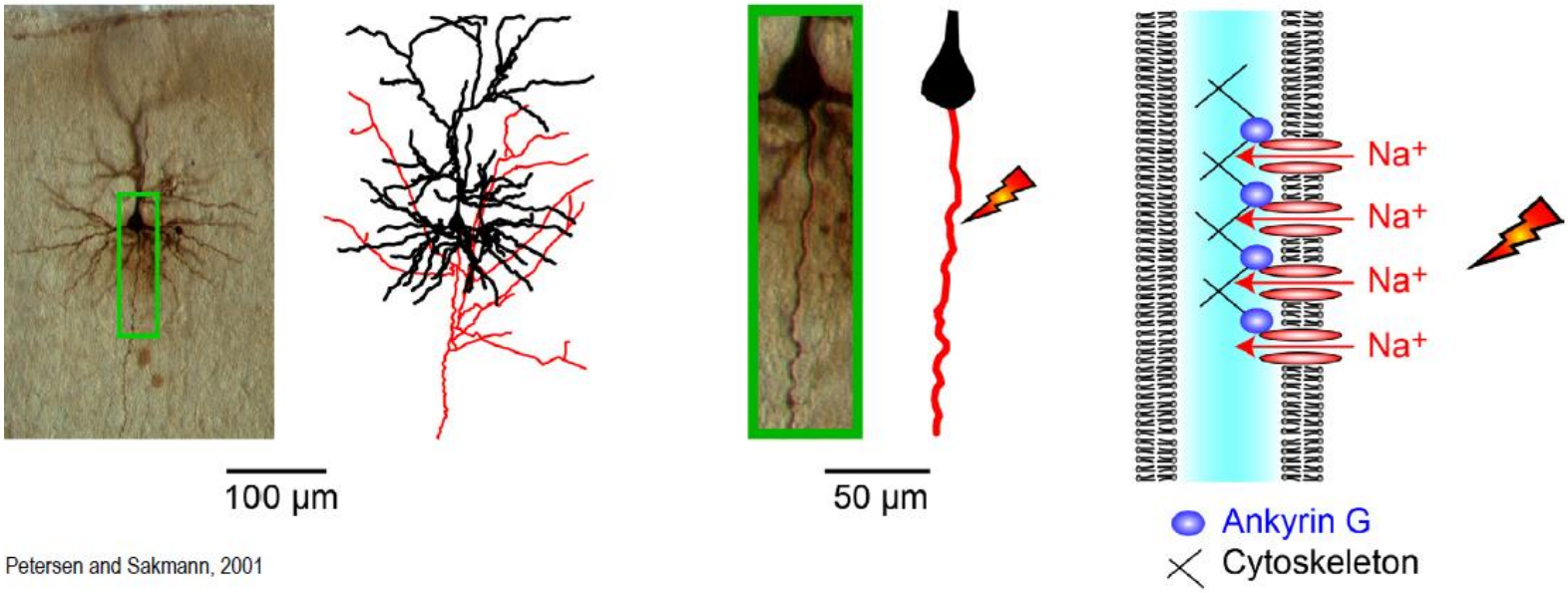


Ionic mechanism of the action potential (in neurons)

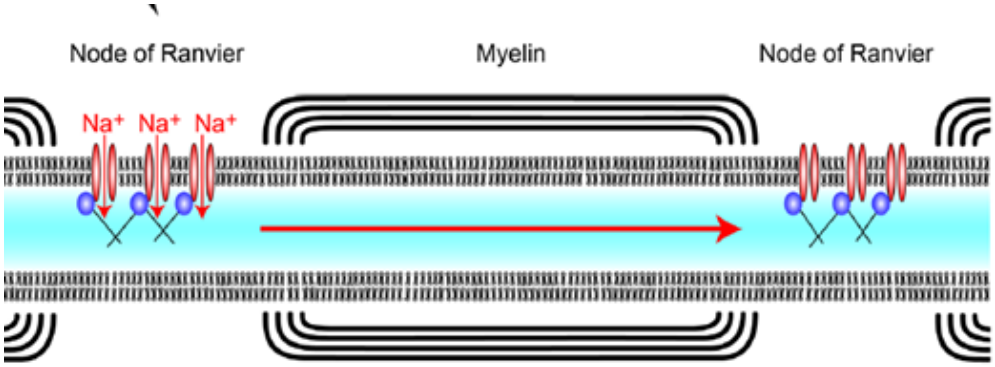
- Stimulus driven depolarization near to the activation threshold (-50 - -40 mV) (few voltage gated Na+ channels will be activated - „local response")
- Rapid depolarization: after reaching the activation threshold (E_m ~ 40 mV) the opening of increasing number of Na^+ - channels produces further depolarization; this activates the remaining voltage sensitive Na+ - channels.
  Positive feed back: auto-amplification similar to a (nuclear) chain reaction!
  Highest gNa^+ is observed short before the peak of the AP
- The activated Na+- channels show rapid inactivation
- Voltage gated K^+-channels are activated with a 0,2-0,3 ms delay (slower opening kinetics)
  gK+ reaches its maximum during the repolarization phase
- Afterpotentials: different types of K+ channels will also be activated

During the AP the ion concentrations in the ICF and ECF do not change significantly – the equilibrium potentials of these ions remain constant!!
(e.g.: IC Na^+ concentration increases only by ~0.013%)

THE AXON INITIAL SEGMENT



Petersen and Sakmann, 2001



Time course of the changes in the ionic conductances during the action potential

If the actual membrane potential (E_m) and ion currents (I_{Na+} and I_{k+}) are known, it is possible to calculate the conductance values for these ions: R=U/I → g=I/U (Ohm's law)

Membrane potential is determined by:

-concentrations of the ions: there is only a small change during the AP

-ion conductance:

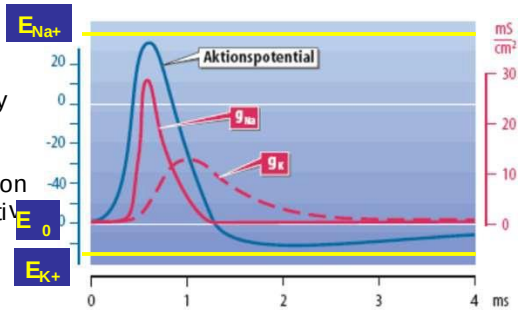
gNa^+ - rapid activation and inactivation

gK^+ - delayed activation – slow inactiv

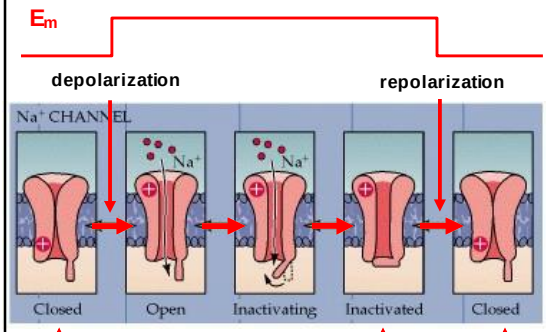
Consequently:

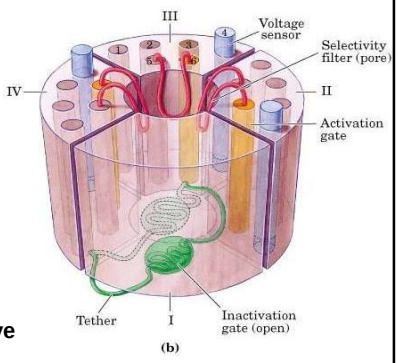
– during the depolarization phase: E_m approaches the equilibrium potential of Na^+ (E_{Na}+ ~+60 mV)

- repolarization: Em approaches the equilibrim potential of K^+ (E_K+~ -70mV)



Functional model of the voltage gated Na+-channel - **Rephracterity** -

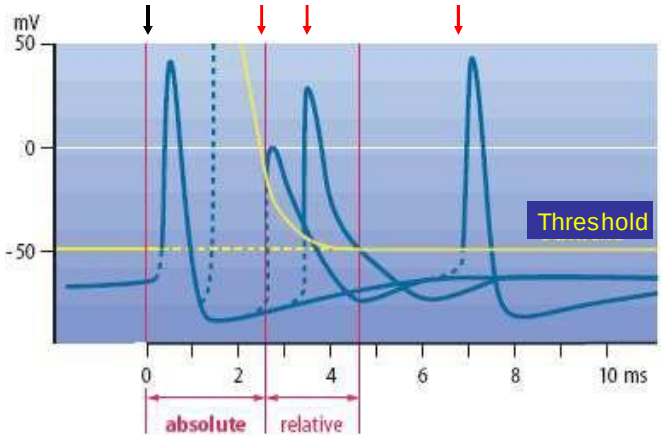




During the AP, the activated Na+ channels become inactivated and they remain in this state until the membrane potential returns again to the resting potential!!

Consequence: **rephractory period**

Time course of the changes in the excitability of the membrane during the AP (rephracterity)

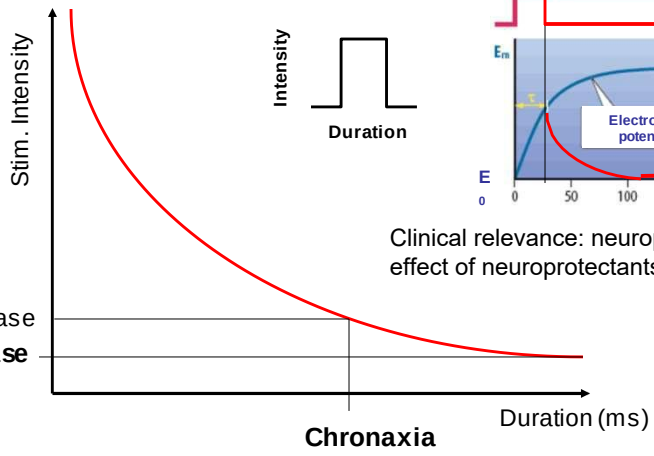


rephractory period

Absolute rephractory period: membrane is completely unexcitable
Relative rephractory period: the activation threshold is elevated (needs stronger stimuli)
Consequence: the frequency of repetitive firing of the neurons is limited (max 500-1000H

Stimulus duration – stimulus intensity function of the excitable membranes

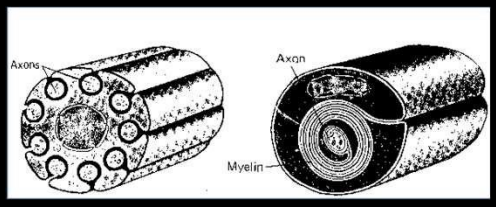
Strength-Duration Curve

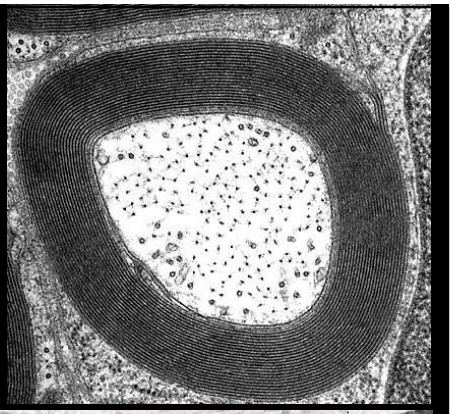


Clinical relevance: neuropathies, effect of neuroprotectants

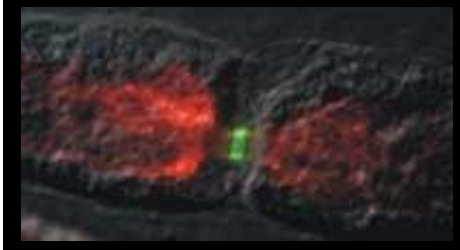
Thick myelinated axons (Aα) are more excitable compared to thin unmyelinated C fibres - both the chronaxia and the rheobase values are lower in Aα fibres!

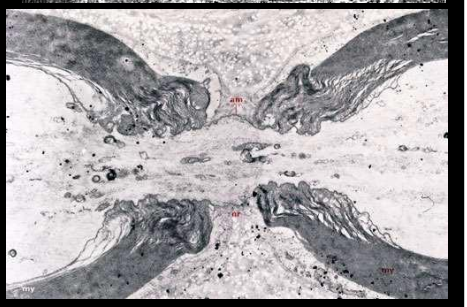
Relation of the Schwann cells and the unmyelinated and myelinated fibres





Na^+_Vchannels: nodal membrane (green)
K^+_Vchannels: paranodal region (red)



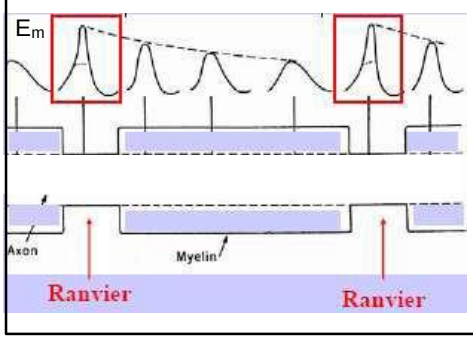


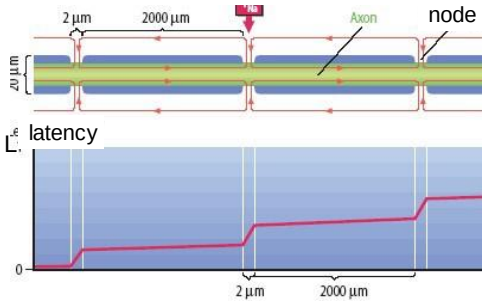
Saltatoric propagation of AP in myelinated axons:

Node of Ranvier (2 μm): development of AP (voltage sensitive Na^+ channels)
Internodium (2-3000 μm): absence of APs!! – electrotonic propagation of depolarization!!
Conduction velocity is low in the nodal region –high in the internodium

Economy: less energy needed to cover Na^+/K^+ ATP-ase activity
(Pathophysiology--- Demyelination)

AP EP EP EP AP





Classifications of the axons of mixed peripheral nerves

Lloyd and Hunt (Sensory)	Erlanger and Gasser (Sensory and Motor)	Diameter (μm)	Velocity (m/s)	Function
Ia fibers	A-alpha fibers	10-20	50-120	alpha motor neurons muscle spindle afferents
Ib fibers	A-alpha fibers	10-20	50-120	Golgi tendon organ
II fibers	A-beta fibers	4-12	25-70	muscle spindle afferents, touch, pressure
III fibers	A-gamma fibers	2-8	15-30	gamma motor neurons
	A-delta fibers	1-5	12-30	touch, pain, temperature
IV fibers	B-fibers	1-3	3-15	preganglionic autonomic fibers
	C-fibers	<1	<2	postganglionic autonomic fibers Sensory: pain, temperature

Comparison of the action potential and the electrotonic potentials

	Action potential	Electrotonic potential
Activation threshold	defined (~-40 mV)	no threshold
Sign of the potential change	always depolarizing	either de- or hyperpolarizing (stimulus dependent)
Amplitude	constant: „all or none" rule	graded (stimulus dependent)
Propagation	without decrement	with decrement
Rephracterity	absolute and relative rephractory period	no rephractory period
Summation	no	temporal and spatial
Mechanism	Voltage dependent ion channels (Na^+, K^+, $Ca2^+$)	passive membrane currents
Biological function	conduction of neuronal signals	Signal conduction and processing (PSPs) Sensorytransduction