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

Measurement: microelectrode + amplifier + voltmeter
-- direct electrical access to the cell

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 the extra- and intracellular fluids:

<table>
<thead>
<tr>
<th>ECF (mmol/L)</th>
<th>ICF (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(interstitial fluid)</td>
<td>(cytosol)</td>
</tr>
<tr>
<td>Na⁺</td>
<td>150</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>100</td>
</tr>
<tr>
<td>K⁺</td>
<td>4.5</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>1.8</td>
</tr>
<tr>
<td>H⁺</td>
<td>0.00004 (pH=7.4)</td>
</tr>
</tbody>
</table>

Plasma membrane

Development of diffusion equilibrium - charge separation - the Nernst potential

Equilibrium: there is no net diffusion

charge separation: cations and anions accumulate near 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 \([\text{ion}]_\text{o}\) (ECF) and \([\text{ion}]_\text{i}\) (ICF) concentrations:

\[
E_{\text{ion}} = \frac{RT}{ZF} \times \ln \frac{[\text{ion}]_\text{o}}{[\text{ion}]_\text{i}}
\]

\[
E_{K^+} = 61.5 \text{mV} \times \log \frac{K^+}{K^+}
\]

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

- \(E_{Na} = 60 \text{ mV} \log 150/15 = +60 \text{ mV}\)
- \(E_{Ca} = 60/2 \text{ mV} \log 1.8/0.0001 = +130 \text{ mV}\)
- \(E_{K} = 60 \text{ mV} \log 4.5/150 = -90 \text{ mV}\)
- \(E_{Cl} = 60/-1 \text{ mV} \log 100/5 = -80 \text{ mV}\)

Further problems:

- Different ions have different equilibrium potentials
- These values also differ from the empirical 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)

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: \(R = \frac{U}{I} \rightarrow I = \frac{U}{R}\) and \(I = U \times g\) \((g=\text{conductance})\)

Electrostatic driving force \((E_i) = ??\)
It is the difference between the actual \(E_m\) and the Nernst potential of the ion:
\(\rightarrow E_i = E_{\text{Nernst}} - E_m\)
Calculation of the net \(K^+\) current: \(I_{K^+} = (E_{K^+} - E_m) \times gK^+\)

\[
\Sigma I_{\text{net}} = 0 = I_{K^+} + I_{Na^+} + I_{Cl^-} = gK^+ \times E_{K^+} + gNa^+ \times E_{Na^+} + gCl^- \times E_{Cl^-}.
\]
**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

\[ E_m = \frac{RT}{F} \ln \left( \frac{P_K [K^+]_a + P_{Na} [Na^+]_a + P_{Cl} [Cl^-]_a}{P_K [K^+] + P_{Na} [Na^+] + P_{Cl} [Cl^-]} \right) \]

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 potential 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

Ratio of the antiport mechanism is 3 Na+ outward pro 2 K+ inward (net 1 + outward/cycl.)
This shifts the calculated \( E_m \) (GHK equation) with cc. -5 mV to the negative direction -hyperpolarising **pump potential**

Consequence:

- 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**!!

![Diagram of sodium-potassium pump](image)

**Equations:**

- A) \( E_m = -65 \text{mV} \)
  - K+ moves from IC to EC
  - Na+ moves from IC to EC

- B) \( E_m = -70 \text{mV} \)
  - 2K+ moves from IC to EC
  - 3Na+ moves from IC to EC

\[ \text{IC} \quad \text{EC} \]
Importance of the membrane capacitance

The electric charges (free ions), 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 is an insulator).

Under resting conditions the membrane capacity determines the number of the charged particles (ions) which can be stored at the given potential difference (Em):

\[ C=\frac{Q}{U} \rightarrow Q=CM \times U_m \quad (U_m=Em) \]

\( C_m \) is a function of cell surface, thickness of the membrane, dielectric constant (physical properties of the membrane components)

Example:

One regular shaped (round) cell, with a diameter of 50 µm at \( E_m=60 \text{ mV} \) with a membrane capacity of \( C_m=1 \text{ µF/cm}^2 \):

calculated number of ions which are stored in this membrane capacitor is:

\[ 30 \times 10^6 \text{ (only 1/200 000 part of the total ions in the ICF!!)} \]

Tonic changes of the membrane potential: electrotone - electrotonic potentials
(“passive” property of the plasmamembrane)

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)

\( \Delta E_m (E_{max}) \) is proportional with the stimulus intensity and the membrane resistance \( (R_m) \)
Extracellular stimulation:

Cathode
– depolarization of the membrane (cathelectrotone)

Anode
– hyperpolarization of the membrane (anelectrotone)

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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activation threshold</td>
<td>no threshold, „obligate“</td>
</tr>
<tr>
<td>Sign of the potential change</td>
<td>either de- or hyperpolarizing (stimulus dependent)</td>
</tr>
<tr>
<td>Amplitude</td>
<td>graded (stimulus dependent) – „analog“ signal</td>
</tr>
<tr>
<td>Propagation</td>
<td>with decrement – local change</td>
</tr>
<tr>
<td>Rephractory period</td>
<td>no refractory period</td>
</tr>
<tr>
<td>Summation</td>
<td>temporal and spatial</td>
</tr>
<tr>
<td>Mechanism</td>
<td>„passive“ membrane currents; opening/closure of ion channels, electrical stimulation</td>
</tr>
<tr>
<td>Biological function</td>
<td>Signal conduction and processing (PSPs)</td>
</tr>
<tr>
<td></td>
<td>Sensory transduction</td>
</tr>
<tr>
<td></td>
<td>Pacemaker activity</td>
</tr>
</tbody>
</table>

## Recordings of passive and active electrical signals in a nerve cell

![Recordings of passive and active electrical signals in a nerve cell](image)

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

**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 feedback:** auto amplification similar to a (nuclear) chain reaction!
  Highest gNa⁺ is observed short before the peak of the AP

- The activated Na⁺- channels inactivate rapidly

- 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 remain constant!!
(e.g.: IC Na⁺ concentration increases only by ~0.013%)

Relative importance of the voltage-gated Na⁺ and K⁺ channels in the development of the (neural) action potential

Control + TTX (Na⁺ blockade) + TEA (K⁺ blockade)

http://www2.neuroscience.umn.edu/eanwebsite/metaneuron.htm
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: \( R = U/I \rightarrow g = I/U \) (Ohm’s law)

Membrane potential is determined by:
- concentrations of the ions: there is only a small change during the AP
- ion conductance:
  - \( g_{Na^+} \) - rapid activation and inactivation
  - \( g_{K^+} \) - delayed activation – slow inactivation

Consequently:
- during the depolarization phase: \( E_m \) approaches the equilibrium potential of \( Na^+ \) (\( E_{Na^+} \sim +60 \text{ mV} \))
- repolarization: \( E_m \) approaches the equilibrium potential of \( K^+ \) (\( E_{K^+} \sim -70 \text{ mV} \))

Functional model of the voltage gated Na+-channel

- Repfractority -

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

**Absolute refractory period**: membrane is completely unexcitable

**Relative refractory period**: the activation threshold is elevated (needs stronger stimuli)

Consequence: the frequency of repetitive firing of the neurons is limited (max. 500-1000 Hz)

Stimulus duration – stimulus intensity function of the excitable membranes

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!
Propagation of electrotonic potentials along elongated membrane structures

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

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

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

Length constant (distance where $E_m = E_{max} \times 0.37$)
- directly proportional to $R_m$
- inversely proportional to $R_a$

Inhomogenous current distribution along the stimulated membrane

Leaky channels

Local circuits model of the conduction of EP
- $R_a$ = axon (length) resistance
- $R_m$ = membrane resistance
Propagation of the action potential

AP is induced by Na\(^+\) influx:
Depolarizes the neighboring axonal segments („local circuit”)

Depolarization is conducted as an **electrotonic potential onto the next axonal segment**

Reaching the activation threshold – Na\(^+\) channels open

Forward propagation:
Membrane regions ahead of the AP have high resistance – large depolarization occurs

Backward propagation is blocked by:
Low membrane resistance (open K\(^+\) channels)
Rephractory state of the Na\(^+\) channels

\[ \text{Squid axon} \]

\[ \text{Membrane current} \]

\[ 20 \text{ m/s} \]

\[ \text{time (ms)} \]

\[ \text{Ra= axon resistance} \]

\[ E_{\text{threshold}} \]

\[ E_0 \]

„Passive” ion fluxes (mostly K\(^+\) currents)

\[ \text{Na}^+ \text{ influx („active” current)} \]

Current density along the axon

Direction of propagation

Potential profile of the propagating depolarization
Conduction velocity (0.5 – 100 m/s) depends on:

- Strength of depolarizing inward currents (activity of $\text{Na}_v^+$ channels – see local anaesthetics)
- 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 $\varnothing$ is ~1mm!!)
  membrane capacitance - (determined by thickness of the membrane)

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

Recording of electric activities of biological membranes using extracellular electrodes

Applications:
ENG: electro-neurography
EMG: electro-myography
ECG: electro-cardiography
EEG: electro-encephalography
ERG: electro-retinography

The potential changes might also be produced by EPs!

a: unipolar recording:
second electrode is „passive“-indifferent
(reference electrode)

b: bipolar recording:
second electrode is „active“—difference signal
Compound action potential of the peripheral nerve

Latency values can be used to determine the conduction velocities of different fibre populations

Classifications of the axons of mixed peripheral nerves

<table>
<thead>
<tr>
<th>Lloyd and Hunt (Sensory)</th>
<th>Erlanger and Gasser (Sensory and Motor)</th>
<th>Diameter (µm)</th>
<th>Velocity (m/s)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia fibers</td>
<td>A-alpha fibers</td>
<td>10-20</td>
<td>50-120</td>
<td>alpha motor neurons</td>
</tr>
<tr>
<td>Ib fibers</td>
<td>A-alpha fibers</td>
<td>10-20</td>
<td>50-120</td>
<td>muscle spindle afferents</td>
</tr>
<tr>
<td>II fibers</td>
<td>A-beta fibers</td>
<td>4-12</td>
<td>25-70</td>
<td>muscle spindle afferents, touch, pressure</td>
</tr>
<tr>
<td>III fibers</td>
<td>A-gamma fibers</td>
<td>2-8</td>
<td>15-30</td>
<td>gamma motor neurons</td>
</tr>
<tr>
<td>IV fibers</td>
<td>A-delta fibers</td>
<td>1-5</td>
<td>12-30</td>
<td>touch, pain, temperature</td>
</tr>
<tr>
<td></td>
<td>B-fibers</td>
<td>1-3</td>
<td>3-15</td>
<td>preganglionic autonomic fibers</td>
</tr>
<tr>
<td></td>
<td>C-fibers</td>
<td>&lt;1</td>
<td>&lt;2</td>
<td>postganglionic autonomic fibers Sensory: pain, temperature</td>
</tr>
</tbody>
</table>
### Comparison of the action potential and the electrotonic potentials

<table>
<thead>
<tr>
<th></th>
<th>Action potential</th>
<th>Electrotonic potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activation threshold</td>
<td>defined (~-40 mV)</td>
<td>no threshold</td>
</tr>
<tr>
<td>Sign of the potential change</td>
<td>always depolarizing</td>
<td>either de- or hyperpolarizing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(stimulus dependent)</td>
</tr>
<tr>
<td>Amplitude</td>
<td>constant: „all or none“ rule</td>
<td>graded (stimulus dependent)</td>
</tr>
<tr>
<td>Propagation</td>
<td>without decrement</td>
<td>with decrement</td>
</tr>
<tr>
<td>Rephracterity</td>
<td>absolute and relative refractory period</td>
<td>no refractory period</td>
</tr>
<tr>
<td>Summation</td>
<td>no</td>
<td>temporal and spatial</td>
</tr>
<tr>
<td>Mechanism</td>
<td>Voltage dependent ion channels (Na⁺, K⁺, Ca²⁺)</td>
<td>passive membrane currents</td>
</tr>
<tr>
<td>Biological function</td>
<td>conduction of neuronal signals</td>
<td>Signal conduction and processing (PSPs)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sensory transduction</td>
</tr>
</tbody>
</table>