Resting potential, action potential and electrical excitability.
Measurement of the membrane potential

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Action potential: Textbook, pages 294-300.
Electrical excitability: Textbook, pages 290-292.
Resting membrane potential

- all living cells exhibit an electrical potential difference between the inner and outer surface of the cytoplasmic membrane

- varies among different cell types, its value is typically between -30 and -90 mV (the electrical potential of the ic. space is always negative compared to the ec. space)
Factors determining the resting potential ($E_m$)

- **Diffusion potential, GHK equation ($E_{\text{diff}}$)**
  - Concentration gradient plus selective permeability
  - Most significant contribution

\[
U = E_m = -\frac{RT}{Fz} \ln \frac{p_K[K]_i + p_{Na}[Na]_i + p_{Cl}[Cl]_o}{p_K[K]_o + p_{Na}[Na]_o + p_{Cl}[Cl]_i}
\]

- **Pump potential ($V_p$)**
  - $Na^+/K^+$ ATP-ase
  - $2K^+/3Na^+$ \(\rightarrow\) electrogenic
  - Depending on the cell type: 2-16 mV (direct contribution)
  - Major contribution to maintaining the concentration gradient across the membrane \(\rightarrow\) indirectly influences $E_{\text{diff}}$

- **Donnan potential**
  - Due to the presence of non-permeable protein anions in the cytosol.
  - Negligible contribution, cells continuously fight against it

\[
= \frac{RT}{zF} \ln \frac{[X^+]^I}{[X^+]^II}
\]
Dependence of the membrane potential on relative ion permeabilities

- at a given ion concentration gradient $E_{\text{diff}}$ is determined by $P_x$

- the greater the permeability for a given ion, the closer the MP will be to the equilibrium (Nernst) potential for that ion. For example, opening of many $K^+$ channels will shift the MP toward -89 mV, while opening of many $Na^+$ channels will shift the MP toward +60 mV.

- the actual MP is the average of the Nernst-potentials of the permeating ions weighted by their permeabilities
Action potential

- change in the membrane potential with a characteristic time course and voltage values
- two major cell types that are able to fire action potentials: nerve- and muscle cells (+ some endocrine cells)
- AP transmits information in neurons and initiates contraction in muscles
- all impulses look alike, similar in shape, amplitude and duration
- strength of stimulus is coded in the frequency
- propagates with constant amplitude
Nomenclature of the action potential (e.g. neural AP)

- depolarization
- overshoot
- repolarization
- hyperpolarizing afterpotential
- subthreshold responses

- stimulus strength
- membrane potential recording electrode
- giant axon of the squid
- stimulating electrode
- membrane potential
- peak
- subthreshold responses
- threshold
- +50
- 0
- -70
- time (ms)

Membrane potential (mV)
The strength-duration curve

- a plot of the threshold current versus pulse duration required to stimulate excitable tissue

- **rheobase** is the minimal current amplitude of infinite duration that results in the depolarization threshold of the cell membranes being reached

- the **chronaxie** is the minimum time over which an electric current (2-fold the strength of the rheobase) needs to be applied to stimulate a muscle fiber or nerve cell

\[
\text{current} \times \text{duration} = \text{constant}
\]

- the capacitive elements (ie. capacitor plates) of the membrane must be charged to reach the necessary threshold potential

- The same charge can be achieved using various combinations of duration and stimulus intensity (these quantities are inversly proportional to each other)
The first direct proof for the Na$^+$-dependence of the action potential (Hodgkin and Katz, 1949)
Measurement of ionic currents: the voltage-clamp principle
(Cole, Hodgkin, Huxley)

\[ I_m = I_i + I_c = I_i + C_m \frac{dV}{dt} \]

\[ \frac{dV}{dt} = 0 \rightarrow I_m = I_i \]
Separation of ionic currents using channel blockers

![Graph showing separation of ionic currents with TTX](image)
time-dependent changes in the Na\(^+\) and K\(^+\) conductance
Permeability changes during an action potential

- typical curves of specific Na$^+$ and K$^+$ conductivity changes during AP
Operation of ion channels during the AP

\[ \Delta E_m \text{ depolarization} \]

- More Na\(^+\) channel opening
- Inactivation of Na\(^+\) ch.
- Delayed opening of K\(^+\) ch.
- Closing of K\(^+\) ch.

\[ P_{Na} \uparrow, I_{Na} \uparrow \]

\[ E_{Na} \]

\[ E_{K} \]

\[ \text{Conductance (mmho/cm}^2) \]

\[ \text{Em} \]

\[ g_N \]

\[ g_K \]

\[ E_m (mV) \]

\[ \text{Time (msec)} \]
Ionic currents during an action potential

- slow depolarization of the membrane to reach the depolarizing threshold
- depolarization opens voltage gated Na\(^+\) channels
- Na\(^+\) influx, which causes further depolarization (\(E_m\) shifts toward the Na\(^+\) equilibrium potential) \(\rightarrow\) more Na\(^+\) channels open \(\rightarrow\) more Na\(^+\) influx \(\rightarrow\) further depolarization ...  
  positive feedback

- the Na channels enter the non-conducting inactivated state which stops the Na\(^+\) current + delayed opening of voltage-gated K\(^+\) channels
- K\(^+\) permeability increases, K\(^+\) ions flow from the cell. K\(^+\) efflux balances Na\(^+\) influx (repolarization)
- Depolarization opens the K\(^+\) channels the K\(^+\) efflux hyperpolarizes the membrane which renders the channels to the closed state  
  negative feedback

- closure of the K\(^+\) channels and the recovery from inactivation of the Na\(^+\) channels result in the reestablishment of the resting potential
Refractory periods

- prevents the fusion of AP and allow the propagation of the separated APs
- the amount of time it takes for an excitable membrane to be ready for a second stimulus once it returns to its resting state
- two types of refractory periods occur during an AP

**absolute refractory period** (ARP): Na⁺ channels are inactivated, no Na⁺ conductance, no new AP can be initiated

**relative refractory period** (RRP): Na⁺ channels have returned to the closed state, but the MP is more negative than the resting MP due to the delayed closing of the K⁺ channels, so stronger depolarization would be needed to reach the firing threshold
Propagation of the AP

- the action potential generated at the axon hillock (cell body) propagates as a wave along the axon with constant amplitude
- the currents flowing inwards at a point on the axon during an AP spread out along the axon, and depolarize the adjacent sections of its membrane
- this depolarization provokes a similar action potential at the neighboring membrane patches
- the rate of propagation is influenced by the thickness of the fibre and the myelinization

the AP propagates towards the synaptic knobs (the axonal termini)
(absolute refractory period due to the inactivation of the Na channels which prevents the backward propagation)
• certain neuronal axons are covered with myelin sheaths
• myelin is a multilamellar membrane that enwraps the axon in segments separated by intervals known as nodes of Ranvier
• increases the conduction velocity of action potentials
• myelin behaves as an insulator, therefore prevents ions from entering or leaving the axon along myelinated segments
• the ionic current from an action potential at one node of Ranvier provokes another action potential at the next node (saltatory conduction)
• some diseases degrade myelin and impair saltatory conduction, reducing the conduction velocity of action potentials (multiple sclerosis)
Measurement of the membrane potential II.

Application of fluorescent dyes/probes for measuring membrane potential.

- Some dyes change their fluorescent properties in an electric field and can therefore be used to follow action potential propagation.
- Another group of dyes does not change its spectroscopic properties in an electric field, however, due to their net negative or positive charge they are distributed across the two sides of the membrane according to their solubility and the electrochemical gradient:
  - Changes in the electrochemical gradient across the cell membrane results in redistribution of the dyes leading to changes in the fluorescence intensity emitted from the cells.
  - The more negative the inside of the cell the more positively charged dye is accumulated within the cell.
  - Negatively charged dye molecules are not accumulated in cells having a negative resting potential, their toxicity is negligible.