Lesson 10

Synapse and Neuromuscular Junction

Objectives

• How depolarization signal transmitted through a synapse

• Quantitative description of a neuromuscular junction
- structure of a neuron
- action potential from a biological perspective
- synapse and ACh receptors

- end-plate potential and Poisson distribution
- exercise

- Ca\(^{2+}\) and Mg\(^{2+}\) influence on the end-plate potential
- post-junctional response to transmitter
- exercise
A typical vertebrate neuron. The arrows indicate the direction in which signals are conveyed. The single axon conducts signals away from the cell body, while the multiple dendrites receive signals from the axons of other neurons. The nerve terminals end on the dendrites or cell body of other neurons or on other cell types, such as muscle or gland cells.
# Ion Concentrations

<table>
<thead>
<tr>
<th>Component</th>
<th>Intra\text{cellular concentration (mM)}</th>
<th>Extra\text{cellular concentration (mM)}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$</td>
<td>5-15</td>
<td>145</td>
</tr>
<tr>
<td>K$^+$</td>
<td>140</td>
<td>5</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>0.5</td>
<td>1-2</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>10$^{-4}$</td>
<td>1-2</td>
</tr>
<tr>
<td>H$^+$</td>
<td>7.2 (pH)</td>
<td>7.4 (pH)</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>2</td>
<td>80</td>
</tr>
</tbody>
</table>
Na\(^+\) and K\(^+\) channels

* voltage-gated Na\(^+\) channels
  - close (at rest Na\(^+\) outside )
  - open (Na\(^+\) enter the cell → further depolarization)
  - inactivated (to reclose although still depolarized → avoid spasma)

* voltage-gated K\(^+\) channels
  - close (at rest K\(^+\) inside )
  - open (efflux of K\(^+\) → to overwhelm Na\(^+\) influx → ready for a new impulse)
  - inactivated (same “ball” mechanism as Na\(^+\) channels)
J. Benitah et al., Biophys. J 73 (1997) 603
(A) An action potential is triggered by a brief pulse of current, which (B) partially depolarizes the membrane, as shown in the plot of membrane potential versus time. The green curve shows how the membrane potential would have simply relaxed back to the resting value after the initial depolarizing stimulus if there had been no Na+ voltage-gated ion channels in the membrane; this relatively slow return of the membrane potential to its initial value of –70 mV in the absence of open Na+ channels occurs because of the efflux of K+ through K+ channels, which open in response to membrane depolarization and drive the membrane back toward the K+ equilibrium potential. The red curve shows the course of the action potential that is caused by the opening and subsequent inactivation of voltage-gated Na+ channels, whose state is shown in (C). The membrane cannot fire a second action potential until the Na+ channels have returned to the closed conformation; until then, the membrane is refractory to stimulation.
(A) The voltages that would be recorded from a set of intracellular electrodes placed at intervals along the axon.
(B) The changes in the Na+ channels and the current flows (orange arrows) that give rise to the traveling disturbance of the membrane potential. The region of the axon with a depolarized membrane is shaded in blue. Note that an action potential can only travel away from the site of depolarization, because Na+-channel inactivation prevents the depolarization from spreading backward. On myelinated axons, clusters of Na+ channels can be millimeters apart from each other.
Schwann Cell

A myelinated axon from a peripheral nerve. Each Schwann cell wraps its plasma membrane concentrically around the axon to form a segment of myelin sheath about 1 mm long. For clarity, the layers of myelin in this drawing are not shown compacted together as tightly as they are in reality.

Figure 11–30 part 1 of 2. Molecular Biology of the Cell, 4th Edition.
Each gap junction (aka nexus junction) contains numerous gap junction channels which cross the membranes of both cells. With a lumen diameter of about 1.2 to 2.0 nm, the pore of a gap junction channel is wide enough to allow ions and even medium sized molecules like signaling molecules to flow from one cell to the next, thereby connecting the two cells' cytoplasm. Thus when the voltage of one cell changes, ions may move through from one cell to the next, carrying positive charge with them and depolarizing the postsynaptic cell.

How?
- voltage gating
- selectivity
- pH gating
- rectification
- …

Gap junction channels are composed of two hemi-channels called connexons in vertebrates, one contributed by each cell at the synapse. Connexons are formed by six 7.5 nm long, four-pass membrane-spanning protein subunits called connexins, which may be identical or slightly different from one another.

from “wikipedia”
Chemical Synapse

When an action potential reaches the nerve terminal in a presynaptic cell, it stimulates the terminal to release its neurotransmitter. The neurotransmitter molecules are contained in synaptic vesicles and are released to the cell exterior when the vesicles fuse with the plasma membrane of the nerve terminal. The released neurotransmitter binds to and opens the transmitter-gated ion channels concentrated in the plasma membrane of the postsynaptic target cell at the synapse. The resulting ion flows alter the membrane potential of the target cell, thereby transmitting a signal from the excited nerve.

Figure 11–33. Molecular Biology of the Cell, 4th Edition.
The binding of two acetylcholine molecules opens this transmitter-gated ion channel. It then maintains a high probability of being open until the acetylcholine has been hydrolyzed. In the persistent presence of acetylcholine, however, the channel inactivates (desensitizes). Normally, the acetylcholine is rapidly hydrolyzed and the channel closes within about 1 millisecond, well before significant desensitization occurs. Desensitization would occur after about 20 milliseconds in the continued presence of acetylcholine.

- ACh concentration lowered by hydrolysis by a specific enzyme (ACh-esterase) in the neuromuscular junction
- *little* selectivity → both K⁺ and Na⁺ (together with some Ca²⁺)
Five homologous subunits (α, α, β, γ, δ) combine to form a transmembrane aqueous pore. The pore is lined by a ring of five transmembrane α helices, one contributed by each subunit. In its closed conformation, the pore is thought to be occluded by the hydrophobic side chains of five leucines, one from each α helix, which form a gate near the middle of the lipid bilayer. The negatively charged side chains at either end of the pore ensure that only positively charged ions pass through the channel. Both of the α subunits contain an acetylcholine-binding site; when acetylcholine binds to both sites, the channel undergoes a conformational change that opens the gate, possibly by causing the leucines to move outward.

Three-Dimensional Image of a Ligand-Gated Ion Channel. The image is based on data obtained from the nicotinic acetylcholine receptor (Unwin, 1993). The views are of the structure (~12nm long) from the side (top) and from above (bottom), looking into the synaptic entrance of the channel. The band across the structure (top) corresponds to the bilayer-spanning region and separates the large extracellular mass (above) from the smaller cytoplasmic mass (below). The tunnel made by the synaptic entrance (bottom) narrows quite abruptly after a length of about 6nm, exposing surfaces where the negatively charged rings at the ends of the M2 segments would be located.

Ribbon diagrams of the whole receptor, as viewed (a) from the synaptic cleft and (b) parallel with the membrane plane. For clarity, only the ligand-binding domain is highlighted in (a) and only the front two subunits are highlighted in (b) (α, red; β, green; γ, blue; δ, light blue). Also shown are the locations of aTrp149 (gold), the MIR and the membrane (horizontal bars; E, extracellular; I, intracellular). The dotted lines on the right denote the three main zones of subunit–subunit contacts. The apex of the C-loop of ad (broken trace in (a)) was not visible in the densities.
Neuromuscular Junction

1) nerve impulse → depolarization → Ca^{2+} channels open → Ca^{2+} inside the neuron terminal → ACh released

2) ACh binds to muscle ACh receptor channels → Na^{+} inside the muscle cell → localized depolarization

3) ACh-induced depolarization → voltage-gated Na^{+} channels open → Na^{+} inside → self-propagating depolarization

4) generalized depolarization → Ca^{2+} channels open in specialized regions (the transverse [T] tubules)

5) Ca^{2+} release channels open → Ca^{2+} from sarcoplasmic reticulum into muscle → contraction of myofibrils
End-Plate Potential (EPP)

- The muscle fiber is stimulated by a motor neuron (Left).

- Arrival of a nerve impulse at the axon terminal of the motor neuron
  - causes acetylcholine to be released into the neuromuscular junction which
  - creates an end plate potential (EPP) in the membrane beneath it (A)
  - but not farther away (B).

- When the EPP reaches the threshold of the fiber (about -50 mv), an action potential is generated that sweeps along the fiber (B)
Miniature EPP (MEPP)

Small fluctuations (typically 0.5 mV) in the resting potential of postsynaptic cells.

They are blocked by *curare* which is known to interfere with the ability of postsynaptic receptors to respond to ACh.

They are the same shape as, but much smaller than the end plate potentials caused by stimulation of the presynaptic cell. Miniature end plate potentials are considered as evidence for the **quantal release** of neurotransmitters at chemical synapses, a single miniature end plate potential resulting from the release of the contents of a single synaptic vesicle.

I.A. Boyd et al., *J. Physiol.* **132** (1956) 74
Binomial Distribution

- large number of vesicles containing ACh
- small probability $p$ of release of any one vesicle

\[ P(x) = \frac{N!}{(N-x)!x!} p^x (1-p)^{N-x} \]

- equally likely ways of realizing $x$ of $N$ release sites
- each configuration is statistically independent

Poisson statistics
Hypothesis

if \( N \to \infty, \ p \ll 1, \ x \ll N \)

\[
\frac{N!}{(N-x)!} = N(N-1)(N-2)...(N-x+1) \sim N^x
\]

\[
(1-p)^{N-x} = (1-p)^{\frac{1}{(N-x)p}}
\]

\[
\lim (1-a)^{1/a} = e^{-1} \ (\text{for} \ a \to 0)
\]

\[
(1-p)^{N-x} \sim e^{-(N-x)p}
\]

\[
\frac{N!}{(N-x)!} p^x \sim (Np)^x
\]

\[m \equiv Np\]
Poisson Distribution

\[ P(x) = \frac{N!}{x!(N-x)!} p^x (1-p)^{N-x} \quad x \in \mathbb{N} \]

\[ \approx \frac{m^x e^{-m}}{x!} \]

"m (= Np)", average quantum release per trial

i) \[ P(0) \approx e^{-m} \approx \frac{n(0)}{N} \]

ii) \[ m = \frac{\text{mean amplitude of EPP}}{\text{mean amplitude of MEPP}} \]

Fig. 10. Comparison of the two methods of determining the mean quantum content of the e.p.p. in the ten experiments of Table 4. Ordinate: \( \log_e [\text{number of nerve stimuli/number of failures of response}] \). Abscissa: [mean e.p.p. amplitude/mean amplitude of spontaneous potentials]. Straight line signifies agreement between the two methods.

I.A. Boyd et al., J. Physiol. 132 (1956) 74
## Exercise - Poisson

<table>
<thead>
<tr>
<th>Quanta released per stimulus</th>
<th>Number of cases observed</th>
<th>Exp. probability</th>
<th>Poisson's value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>44</td>
<td>0.22</td>
<td>0.25</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>0.27</td>
<td>0.27</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>0.19</td>
<td>0.126</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>0.06</td>
<td>0.036</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>0.025</td>
<td>0.012</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 8. Histograms of e.p.p. and spontaneous potential amplitude distributions in a fibre in which neuromuscular transmission was blocked by increasing the magnesium concentration of the Krebs's solution to 12.5 mm. Peaks in e.p.p. amplitude distribution occur at 1, 2, 3 and 4 times the mean amplitude of the spontaneous miniature potentials. Gaussian curve is fitted to spontaneous potential distribution and used to calculate theoretical distribution of e.p.p. amplitude (continuous curve). Arrows indicate expected number of failures of response to nerve stimuli.
Effect of Ca$^{2+}$ and Mg$^{2+}$ - 1

...on the transmitter release

\[ \text{Ca} + X \rightleftharpoons \text{CaX} \quad (K_1, \text{diss.}) \]
\[ \text{Mg} + X \rightleftharpoons \text{MgX} \quad (K_2, \text{diss.}) \]

\[
\begin{align*}
[X_0] &= [X] + [\text{CaX}] + [\text{MgX}] \\
[X] &= [X_0] - [\text{CaX}] - [\text{MgX}] \\
[\text{MgX}] &= [X_0] - \frac{K_1[\text{CaX}]}{[\text{Ca}]} - [\text{CaX}] \\
K_1[\text{Mg}[\text{CaX}]] &= K_2[X_0][\text{Ca}] - K_2[\text{Ca}][\text{CaX}] - K_1K_2[\text{CaX}] \\
\end{align*}
\]

\[ K_1 = \frac{[\text{Ca}][X]}{[\text{CaX}]} \quad K_2 = \frac{[\text{Mg}][X]}{[\text{MgX}]} \]

\( X \rightarrow \text{presynaptic structure} \)
Effect of Ca\(^{2+}\) and Mg\(^{2+}\) - 2

\[
[\text{CaX}] = K_1[M\text{g}] + K_2[\text{Ca}] + K_1K_2 = K_2[X_0][\text{Ca}]
\]

\[
[\text{CaX}] = \left( \frac{[X_0]}{K_1} \right) \frac{[\text{Ca}]}{1 + \frac{[\text{Mg}]}{K_2} + \frac{[\text{Ca}]}{K_1}}
\]

EPP amplitude = \(k[\text{CaX}]^n\)

\[
\frac{1}{\sqrt[4]{\text{EPP}}} = \frac{1}{K_1W^{1/4}k} + \frac{1}{W^{1/4}k} \left( \frac{[\text{Mg}]}{K_2} + 1 \right) \frac{1}{[\text{Ca}]}
\]

where \(W = \frac{[X_0]}{K_1}\)

---

...on the transmitter release

Ca → stimulating

Mg → inhibiting

---

F.A. Dodge et al., J. Physiol. 193 (1967) 419
Although AChR do not form separate pathways for Na and K, the two ions move independently through AChR channels → the synaptic conductance and reversal potential can be represented by separate conductances and driving potentials.
from the conservation of the current

\[ V_m = \frac{g_r E_r + g_{Na} E_{Na} + g_K E_K}{g_r + g_{Na} + g_K} \]  (Cl playing no role)

with the simplifying assumption

\[ g_{Na} = g_K = g_s / 2 \]

\[ E_{Na} + E_K \equiv 2E_s \]

\[ V_m = \frac{g_r E_r + g_s E_s}{g_r + g_s} \]

if \( g_r \sim g_s \rightarrow depolarization \) because \( E_s \) more positive than \( E_r \) because of \( E_{Na} \)
Exercise

For $g_{Na} = g_{K}$ in the end-plate region activated by Ach and with $E_{K} = -95 \text{ mV}$ and $E_{Na} = 50 \text{ mV},$

a) what is the reversal potential?

b) if $g_{Na}/g_{K}=1.29$, what is the reversal potential?

\[ V_{m}^{\text{rev}} = \frac{g_{Na} E_{Na} + g_{K} E_{K}}{g_{Na} + g_{K}} \]  

(Cl playing no role)

\[ = \frac{50 - 95}{2} = -22.5 \text{ mV} \]

\[ V_{m}^{\text{rev}} = \frac{g_{Na} E_{Na} + g_{K} E_{K}}{g_{Na} + g_{K}} \]

\[ = \frac{1.29 \times 50 - 95}{2.29} = -13.3 \text{ mV} \]
To be retained…

- axons and dendrites
- function of Na$^+$ and K$^+$ channels for the action potential
- synapse $\rightarrow$ ACh receptors
Literature
