I. Introduction

Fig. 1 Composition and size of cells and organelles (Ganong, 21st edition)

Water - 70-85% of cell mass
Electrolytes - (e.g. K⁺, Na⁺, Cl⁻, Ca²⁺, Mg²⁺, HCO₃⁻) <1% of cell mass
Proteins (amino acids) - 10-20% of cell mass
Lipids - 2-3% cell mass
Carbohydrates - 1%, mostly in combination with proteins & lipids as glycoproteins & glycolipids
Nucleic acids - nucleotides

Fig. 2 Size of cells and organelles (Vander Physiology)
I. Structure of the cell membrane

A. Plasma membrane - the fluid-mosaic model (Fig. 3)

1. The cell membrane is generally thin, 7.5-10 nm, and elastic. Protein 55%, phospholipid, 25%, cholesterol 13%, other lipids 4%, carbohydrates, 3%.

2. The membrane is a phospholipid bilayer with proteins embedded and cholesterol woven in. The bilayer is responsible for passive permeability properties of the membrane.

3. Lipids decrease movement of most molecules through the membrane, proteins provide pathways for selective entry.

   a. The phospholipids are amphipathic. They consist of a phosphorylated glycerol backbone (head) that is hydrophilic and two nonpolar fatty acid chains that are hydrophobic.

      1. The hydrophilic ends are oriented toward the exterior of the membrane, facing extracellular fluid (ECF), and intracellular fluid (ICF).

      2. The hydrophobic ends move away from water and attract each other; they are in the center of the membrane.

   b. Integral and peripheral protein functions

      1. Integral proteins, embedded in the membrane, form receptors, ion (charged particle) channels, pumps and carriers, and enzymes. They are anchored to the membrane by hydrophobic interactions.
2. Peripheral proteins – loosely attached by electrostatic interactions with
integrated proteins, roles in cellular communication, e.g. regulatory
protein subunits for ion channels and transmembrane receptors.
c. The cell membrane is fluid due to the lipids, and the proteins are
globular masses that float in the lipids. Cholesterol is a “fluidity buffer” that
diminishes lateral membrane mobility. It also reduces permeability to
small water-soluble molecules.
d. Lipid rafts: (cholesterol and sphingolipids) form micro (up to 200 nm)
domains that are gel-like, called lipid rafts, where proteins are secured, and
specific types of signaling will occur.
4. Carbohydrates join via covalent bonds with many of the integral proteins in the
membrane to form glycoproteins, and with lipids to form glycolipids to form the
glycocalyx – cell-cell contact, recognition (immune function) and
communication.

B. Membrane junctions between cells (Fig. 4)
1. Tight junctions - fusion of the plasma membranes of two adjacent cells.
   A selective barrier - also called zonulae occludens.
2. Desmosomes - spot welds formed by protein filaments (intermediate). No
   transcellular communication - also true of zonulae adherens, and of
hemidesmosomes, and focal adhesions that attach cells to basal lamina
3. Gap junctions - proteins-units (connexons) from two adjacent cells form channels
   for passage of ions and other small molecules between the cells.

C. Cell Adhesion Molecules (CAMS) – sticky proteins on the cell surface that bind with other cells
or the extracellular matrix. They govern cell-to-cell interactions and are necessary for embryonic
development, cell growth and differentiation, pathogen detection, inflammation, and wound repairs.
CAMs bind to like molecules on other cells (homophylic binding), to other molecules (heterophylic)
or to laminins in the extracellular matrix.
1. Calcium dependent CAMS
   a. Integrins – heterodimers that bind to various laminins, IgGs,
   b. Cadherins Ca\(^{2+}\) dependent molecules, cell to cell in homophilic relations
   c. Selectins (LEC-Cams) - lectin-like domains that bind to carbohydrates (mucins on WBC)

2. Calcium independent CAMs
   a. Ig superfamily of immunoglobins (later section on immunology)
   b. Lymphocyte homing receptors

II. Body fluid compartments
   A. Fluid, or "total body water" is about 50-70% of body weight
      1. Extracellular fluid (ECF) is 20% of body weight
         a) Interstitial 15% (extravascular - outside the vessels)
         b) Intravascular 5% (blood plasma)
      2. Intracellular fluid (ICF) is 40% of body weight.
   B. Electrolyte composition of intra- and extracellular fluids - (Table 1).
   C. Solid components, 25%: protein (18%), minerals (7%) and fat tissue (15%)

Table I: Electrolyte composition of intra- and extracellular fluid (Costanzo)

<table>
<thead>
<tr>
<th>Substance and Units</th>
<th>Extracellular Fluid</th>
<th>Intracellular Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^{+}) (mEq/L)</td>
<td>140</td>
<td>14</td>
</tr>
<tr>
<td>K(^{+}) (mEq/L)</td>
<td>4</td>
<td>120</td>
</tr>
<tr>
<td>Ca(^{2+}), ionized (mEq/L)</td>
<td>2.5(^{+})</td>
<td>1 \times 10(^{-4})</td>
</tr>
<tr>
<td>Cl(^{-}) (mEq/L)</td>
<td>105</td>
<td>10</td>
</tr>
<tr>
<td>HCO(_3^{-}) (mEq/L)</td>
<td>24</td>
<td>10</td>
</tr>
<tr>
<td>pH(^{+})</td>
<td>7.4</td>
<td>7.1</td>
</tr>
<tr>
<td>Osmolarity (mOsm/L)</td>
<td>290</td>
<td>290</td>
</tr>
</tbody>
</table>

\(^{+}\)The major anions of intracellular fluid are proteins and organic phosphates.
\(^{+}\)The corresponding total [Ca\(^{2+}\)] in extracellular fluid is 5 mEq/L or 10 mEq/dL.
\(^{+}\)pH is -log\(_{10}\) of the [H\(^{+}\)]; pH 7.4 corresponds to [H\(^{+}\)] of 40 \times 10\(^{-9}\) Eq/L.

D. Total solute concentration (osmolarity) is the same in ECF and ICF

III. Functions of plasma membranes
   A. Regulate the passage of substances into and out of the cell
      1. Across the membrane through channels, by diffusion or via carriers or pumps
      2. Endocytosis (into the cell by engulfing) and exocytosis (out of the cell – release of substances from vesicles that fuse to the membrane)
   B. Detect chemical messengers arriving at the cell surface (receptors)
   C. Link adjacent cells together via membrane junctions and CAMs
   D. Anchor proteins
IV. Transport across (through) the cell membrane

A. Membrane Permeability - Introduction

Membranes are "selectively" permeable - allowing some substances, but not others to pass through. There are several mechanisms by which substances can pass through membranes. These mechanisms fall in three major categories: diffusion (due to random thermal motion), osmosis (flow of water across a semipermeable membrane due to differences in solute concentration) and protein-mediated processes (channels, pumps, carriers). Water movement across membranes will occur either via movement between phospholipids or via water channels (Aquaporins).

Fig. 5A - Examples of channels and transporters

![Diagram of transport mechanisms](image)

Fig. 5B – Simple diffusion, in the absence of pumps and channels necessary for charged particles

Aquaporins – H₂O
Aquaglyceroporins gases glycerol small uncharged polar molecules
B. Diffusion - passive movement

A. Simple diffusion – When starting with a higher concentration of a substance in one of two compartments, net diffusion (net flux) will move the substance downhill from the higher to the lower concentration. Net rate of diffusion (J, moles or gm per sec)) is the difference between movement in both directions: J is proportional to the permeability (P) and the surface area of the membrane (A, cm²). \((C_A - C_B)\) is the difference in concentration on the two sides of the membrane (mole/cm³).

\[
\text{NET FLUX} = J = PA(C_A - C_B) \quad \text{(Costanzo)}
\]

Permeability (P) includes the partition coefficient (lipid solubility, K), the diffusion coefficient within the membrane in cm²/sec (D), and membrane thickness (cm, \(\Delta x\))

\[
P = KD/\Delta x \quad \text{Note that the permeability and therefore, net flux, are inversely proportional to the thickness of the membrane that solute will cross.}
\]

1. Factors that influence the diffusion coefficient (D) for a particular molecule:
   a. Size of the molecule - large molecules such as glucose do not pass through the membrane as easily as smaller molecules. D is inversely proportional to the molecular radius of the solute.
   b. Viscosity of the medium – D is inversely proportion to the viscosity of the medium
   c. Temperature - higher temperature leads to greater thermal motion of molecules and more net flux.
   d. Kinetic energy – Boltzman’s constant for the solute

The description thus far assumed that the solute is a nonelectrolyte.

2. For charged ions (or electrolytes), an electric potential difference across the membrane also will cause diffusion of ions. Therefore it is important to consider both the concentration difference and the electrical difference. In addition, the diffusion generates a potential difference across a membrane, called a diffusion potential.

3. Presence of channels - charged ions such as Na⁺, K⁺ diffuse at faster rates than their low solubility in lipids would predict. These are four ways in which channels can be "gated" or “activated”:
   a. Ligand or receptor-activated - the channel opens when a specific chemical binds to the channel protein outside the cell
   b. Second messenger-activated – gated by intracellular signaling molecule, e.g. cAMP.
   c. Voltage-activated - the channel opens when a "threshold voltage" is reached
   d. Mechano-sensitive - the channel opens when the channel protein is stretched or pressure is applied
Fig. 5 C. Artificial membrane between cells that allows passive diffusion of glucose shows that the concentration on the two sides of the membrane will equilibrate, but flux will continue.

C. Saturation

Facilitated diffusion (transport) (Fig. 6). A transport (carrier) protein facilitates (assists) diffusion of the substance across the membrane. The rate of facilitated transport will saturate, i.e. it will reach a maximal flux (Vmax), called transport maximum TM, when all of the carrier sites are occupied. For simple diffusion, there is no Vmax. The binding sites on transport proteins are stereospecific, but competition can occur when closely related substances bind to the sites and inhibit transport.

IV. Primary active transport - uses energy (ATP) to move (pump) ions across membranes (Fig. 7) and like facilitated diffusion, it is a form of mediated transport
A. Transport that is powered by phosphorylation of a transport protein
   1. Hydrolysis of ATP
   2. Uphill movement of ions against the concentration gradient (from low to high concentration)
   3. The Na\(^+\)-K\(^+\) ATPase pump - maintains concentration gradients in virtually every cell. [Na\(^+\)] is high outside, [K\(^+\)] is high inside the cell. The pump is a protein that sends 3 Na\(^+\) out for every 2 K\(^+\) that it sends into the cell.
      a. The pump is electrogenic since there is a net change in charge which causes a flow of current.
   4. The Ca\(^{2+}\) ATPase: Ca\(^{2+}\) is kept very low inside the cell by plasma membrane ATPase (PMCA), also Ca\(^{2+}\) ATPase on endoplasmic reticulum and sarcoplasmic reticulum in skeletal muscle (SERCA).
   5. H\(^+\)-K\(^+\) ATPase - transports H\(^+\) out of cells (e.g. acid secretion in stomach)
B. H\(^+\)-ATPase that acidifies intracellular organelles, e.g. Golgi complex
C. H\(^+\)-ATPase on mitochondrial inner membrane - generally synthesizes ATP using energy of H\(^+\) gradient across membrane

Fig. 7A. Na\(^+\)-K\(^+\) ATPase (pump) In one cycle of the pump, in the E1 state, 3 Na\(^+\) ions bind to the high affinity binding region inside the cell, and one ATP molecule is hydrolyzed to ADP + Pi releasing energy. A conformational change occurs, and the enzyme switches to E2 state in which affinity for Na\(^+\) is low, and 3 Na\(^+\) ions are released to the ECF. Then, 2 K\(^+\) ions bind to high affinity binding sites for K\(^+\) and Pi is released. The enzyme binds ATP, and 2 K\(^+\) ions are released to the ICF. One charge (net) flows out of the cell for each pump cycle. (1) Na\(^+\)-binding site, (2) K\(^+\)-binding site, (3) ouabain (cardiac glycoside) binding site, (4) phosphorylation site, (5) ATP-binding site (Ganong, Fig. 1-32, 23rd edition).

Fig. 7B. Sodium and potassium are moved against electrochemical gradients
V. Secondary active transport - due to the concentration gradient (e.g. in Na\(^+\)) created by primary active transport, the ion flows across the membrane from the side with the high concentration of the ion to the side with the low concentration

A. Na\(^+\) may allosterically modify affinity of a transport protein for its substrate.

B. Co-transport (symport) - Na\(^+\) runs down its concentration gradient and goes into the cell, and it takes for example, Cl\(^-\), sugar or amino acids into the cell - via a carrier in the membrane.

C. Counter transport (antiport or exchange) - Na\(^+\) runs down its concentration gradient into the cell, and H\(^+\) or Ca\(^{++}\) for example (see Figs. 8-9) is moved out of the cell.

Fig. 8

VI. Measures of solute concentration are:

A. Molarity - A one molar solution contains one mole (6 x 10\(^{23}\) molecules) of a substance. It is the molecular weight (in gms), placed in a liter of water.

B. Equivalent - takes the valence (charge) of ionized substances into account. One mole of Ca\(^{2+}\) = 2 equivalents of Ca\(^{2+}\)

C. Osmolarity - moles of solute particles per liter (volume) of solution. One mole of NaCl contains about 2 osmoles because there are both Na\(^+\) and Cl\(^-\) particles. Therefore a 1 molar solution of NaCl is 2 osmolar. (*osmotic coefficient of NaCl about 0.93 - for accurate calculation, multiply 0.93 x 2 osmoles) that takes the osmotic coefficient into account (compound, concentration, temperature)
D. Osmolality - 1 mole per kg (based on mass). For plasma, osmolarity is 1-2% less than osmolality.

E. Percent solution - Sometimes solutions are described according to the number of grams in 100 ml. A ten percent solution of glucose contains 10 gms in 100 ml total fluid.

Example:

NaCl - 154 mM (~308 mOsm) is equal to about a 0.9% solution (isotonic)
(molarity - molecular wt in gm/L)

- Na 23 gm
- Cl 35 gm
- NaCl 58 gm

To be worked out on the board in class.

VII. Osmosis (movement of water)

The movement of solvent molecules (i.e. water) across a semipermeable membrane due to differences in solute concentration which creates osmotic pressure.

A. Osmotic pressure - the pressure that must be applied to stop osmosis when it is due to a solution containing nonpenetrating solutes on one side of a membrane separated by a semipermeable membrane from pure water on the other side. Osmotic pressure can be measured with an osmometer (freezing point.)

van't Hof equation: \[ \pi = gC \sigma RT \]

- \( \pi \) = osmotic pressure (atm or mm Hg)
- \( g \) = number of particles per mole solution (Osm/mol)
- \( C \) = concentration (mmol/L)
- \( \sigma \) = reflection coefficient (0-1) freely permeable to impermeable
- \( R \) = gas constant (0.082 L- atm/mol - K)
- \( T \) = absolute temperature (K)

B. Isotonic solutions with respect to plasma - have the same osmolality as plasma (and extracellular fluid), which is almost 300 mOsm. Isotonic solutions are iso-osmotic with plasma.

1. Tonicity is based only on non-penetrating particles; it takes the properties of the membrane into account. Tonicity causes steady state volume changes in cells.

   a. Hypertonic solution - cells shrink in a hypertonic extracellular solution when water leaves the cell
   b. Hypotonic solution - cells swell (water enters the cell) in a hypotonic extracellular solution.
   c. Isotonic solution - cell volume is unchanged in an isotonic solution

2. Osmolarity is based on all particles regardless of whether they are penetrating or non-penetrating. It therefore is independent of membrane characteristics.
VIII. Endocytosis and exocytosis (Fig. 11)

A. Endocytosis - an active process in which plasma membranes form pockets that engulf extracellular fluid and other material that pinch off and enter the cell.
   1. Pinocytosis - fluid adsorption
   2. Phagocytosis - "cell-eating"  Example: polymorphonuclear leukocytes in blood engulf bacteria (also see Fig. 12)
   3. Clathrin-mediated (receptor-mediated) endocytosis

B. Exocytosis - opposite of endocytosis - pockets contain material that leaves the cell
   1. Insert new plasma membrane
   2. Release membrane-impermeant molecules synthesized by the cell.

Fig. 11. Protein processing by the Golgi apparatus, secretion by exocytosis and membrane recovery by endocytosis

(Ganong, Fig. 1-24)
Exocytosis and Endocytosis

Fig. 12. Example of phagocytosis in the eye by an epithelial cell: The rod and cone photoreceptors shed and are phagocytosed by the retinal pigment epithelium (RPE). The RPE is a monolayer of epithelial cells between the photoreceptors and the choroidal blood circulation behind it. The RPE forms the blood-retina barrier for that circulation.
Lecture 2. The nervous system, the neuron; genesis of the membrane potential

I. The nervous system (sensory detection, information processing, behavior)

A. Central nervous system (CNS) (neurons and glia cells)
   1. Brain
   2. Spinal cord

B. Peripheral nervous system (PNS)
   1. Afferent - signals from tissues and organs to the CNS
   2. Efferent - signals to effector cells and ganglia
      a. Somatic NS - to skeletal muscles; voluntary
      b. Autonomic NS - to smooth and cardiac muscles and glands, involuntary.
         Sympathetic, parasympathetic and enteric

II. Neurons (nerve cells) - general structure (Fig. 1B)

1. Dendrites
2. Cell body
3. Axon hillock
   Then: Axon initial segment
4. Axon terminal

A. Cell body (soma) - Contains nucleus and most other organelles
B. Dendrites - Receive incoming signals via synapses with other neurons - main organelles are microfilaments and microtubules
   Sensory neurons such as photoreceptors in the eye, or mechanoreceptors in the skin don't have dendrites with neurotransmitter receptors, they have receptors for physical
stimuli in specialized regions (e.g. the outer segments in photoreceptors transduce light to neural signals).

C. Axon - carries outgoing neural signals, and also transport proteins and polypeptides made in the cell body to terminal (orthograde), and neurotrophins such as nerve growth factor from terminal where they are taken up, to cell body (retrograde). The axon generally does not have rough ER, Golgi bodies, or ribosomes – however, it contains smooth ER and prominent microtubules involved in axonal transport. Transport can be as fast as 400 mm/day.

III. Genesis of membrane potentials
A. The membrane potential is the voltage difference that exists across the plasma membrane of a cell (see Fig. 2). When the cell is at rest, and at the resting (membrane) potential, the inside of the cell is always negatively charged with respect to the outside (-5 to -90 mV). Membrane potentials can be measured directly by placing very fine-tipped “sharp” recording electrodes inside cells and referencing to a point outside the cell, or by using a patch pipette sealed to a membrane and referenced to a point outside the cell.

B. Hyperpolarizing - more negative than the resting potential
2. Depolarizing - more positive than the resting potential.
3. Repolarizing - a change toward the resting potential, from more positive or more negative.

C. The resting potential is determined by:
1. The difference in ion concentrations [ion] of the intra- and extracellular fluid. Recall that [K⁺] is very high inside the cell, [Na⁺] is high outside the cell, due to the Na⁺-
K⁺ ATPase pump. This difference in intra- and extracellular ion concentrations can be used to determine the equilibrium potential, or diffusion potential, for each ion:

a. The equilibrium potential for a given ion is the membrane potential for which the electrical force is equal and opposite to the concentration force. Thus the given ion is at electrochemical equilibrium.

\[
E_{\text{ion}} (\text{mV}) = -60 \times \log \left( \frac{[\text{ion conc}]_{\text{in}}}{[\text{ion conc}]_{\text{out}}} \right) (2.3RT/zF) = -60 (\text{+ ion}) + 60 (\text{- ion})
\]

\[
K^+ \quad E_{K^+} = -60 \log \left( \frac{120}{4} \right) = -60 \times 1.5 = -90 \text{ mV}
\]

\[
Na^+ \quad E_{Na^+} = -60 \log \left( \frac{14}{140} \right) = -60 \times -1 = +60 \text{ mV (often reported as +65)}
\]

\[
Cl^- \quad E_{Cl^-} = +60 \log \left( \frac{10}{105} \right) = -60 \times 1.02 = -61.2 \text{ mV (sometimes about -90)}
\]

-60 is a constant that depends on the gas constant, the Faraday number (F), the valence (z), and the absolute temperature (T). At room temperature the constant is 58 (frogs); at body-temperature for warm-blooded animals, it is 61 (about 60).

Note that if the concentrations were equal inside and outside the cell for an ion, then \(E_{\text{ion}}\) would be zero (the log of 1 = 0)

b. The equilibrium potential in millivolts (mV) can be calculated with the Nernst equation:

Note that Ca²⁺ concentration is 4 orders of magnitude lower in ICF than in ECF

2. The second important factor for the resting potential is the permeability \(p\) or in electrical terms, conductance \(g\) of the plasma membrane to the different ions. If channels are open so that the ion can flow down the chemical concentration gradient created by a pump, then
the membrane potential can approach the $E_{ion}$. *For a neuron at rest, its membrane potential is
generally much nearer the $E_{K^+}$ than $E_{Na^+}$ because more $K^+$ channels are open and $K^+$ can flow
out of the cell and cause the cell to approach $E_{K^+}$ which is -90 mV (calculated from Table 1). $E_{Na^+}$ is +60 mV. If the conductance for $Na^+$ were to increase, $Na^+$ would flow into the cell and the
inside of the cell would become more positive. Thus, altering the relative conductances will move
the membrane potential between the limits set by $E_{Na^+}$ and $E_{K^+}$. This relationship is described
by the:

**chord conductance equation**

$$Em = \frac{g_{K^+}}{g_{Na^+} + g_{K^+} + g_{Cl^-}} E_{K^+} + \frac{g_{Na^+}}{g_{Na^+} + g_{K^+} + g_{Cl^-}} E_{Na^+} + \frac{g_{Cl^-}}{g_{Na^+} + g_{K^+} + g_{Cl^-}} E_{Cl^-}$$

3. The **Goldman/Hodgkin/Katz** equation:

$$E_{m,Na\rightarrow Cl}^{K} = \frac{RT}{F} \ln \left( \frac{P_{Na^+}[Na^+]_{out} + P_{K^+}[K^+]_{out} + P_{Cl^-}[Cl^-]_{in}}{P_{Na^+}[Na^+]_{in} + P_{K^+}[K^+]_{in} + P_{Cl^-}[Cl^-]_{out}} \right)$$

This equation predicts the membrane potential based on several ions, and their
concentrations inside and outside of the cell. The equation says that when a membrane is
permeable to several different ions, the resting membrane potential depends on permeability,
charge, and concentrations of all of the ions. So, the resting potential is not at the $E_{ion}$ for any
specific ion, but can be in a steady state, a standoff position between equilibrium potentials of the
different ions. Note the effect of valence – $Na^+$ vs $Cl^-$. To sum up so far, the resting potential is mainly influenced by these two factors.

a. The difference in ion concentrations (due to the $Na^+-K^+$ ATPase pump)

b. Ion permeability: $K^+$ conductance is 50 to 75 times more than $Na^+$ conductance at
rest, and this sets the resting membrane potential much nearer to $E_{K^+}$ than to $E_{Na^+}$

Two additional factors that contribute to the resting potential of the membrane:

c. The $Na^+-K^+$ ATPase pump contributes directly to the resting potential. Since the
pump sends three $Na^+$ ions outside the cell for each 2 $K^+$ ions that it brings in, there is a loss of
one positive charge (+) from the cell. Thus the pump it is said to be electrogenic. It contributes
about -4 mV to the resting potential of a muscle cell.

d. There are negatively charged proteins (anions) in the cell that cannot move through
the cell membrane (causing the Donnan Effect – see below). They contribute to the osmotic
pressure of the cell as well.

IV. Gibbs - Donnan Effect  (only responsible for highlighted portion – NOT THE EQUATIONS)

Donnan (and Gibbs) showed that in the presence of nondiffusible (impermeant) ions, such as
negatively charged proteins inside cells, the diffusible ions distribute themselves so that at equilibrium the diffusible ions are at electrochemical equilibrium: their concentration ratios are equal. Theoretically, the KCl inside and outside a cell that contains nondiffusible anions is:

\[
\frac{[K^+]_o}{[K^+]_i} = \frac{[Cl^-]_i}{[Cl^-]_o} \quad \text{or} \quad [K^+]_o \times [Cl^-]_o = [K^+]_i \times [Cl^-]_i
\]

Achieving the Donnan equilibrium creates an osmotic imbalance, with more particles on the side that has the impermanent anion (see Fig. 3 on next page). This could be dangerous for cells – they would swell due to osmosis if they had more particles in intracellular fluid than extracellular fluid.

In practice in neurons, the Na\(^+\)-K\(^+\) ATPase pump maintains a concentration gradient that keeps the osmotic imbalance caused by the Donnan "Equilibrium" from prevailing. The Donnan effect is quite important when we consider colloid osmotic pressure caused by proteins in blood vessels.

Before the Donnan equilibrium is established:
Y\(^-\) is a large nondiffuable anion (protein).
K\(^+\) and Cl\(^-\) will diffuse freely

After the Donnan equilibrium is established:
\[
[K^+]_o \times [Cl^-]_o = [K^+]_i \times [Cl^-]_i
\]

Total positive and negative charges in each compartment are in balance, but there are more particles in compartment A.
Pathophysiology note: When the Na\(^+\)-K\(^+\) ATPase pump cannot operate properly and maintain the normal ionic gradient, due for example to lack of oxygen, cell injury will occur, partially because the cells swell. Lack of oxygen (hypoxia) in tissue can occur as a consequence of lack of blood flow (ischemia), perhaps due to a blocked blood vessel. The local tissue can die if circulation is not restored; the type of cell death that occurs is called necrosis. This is what happens in a heart attack (myocardial infarction, MI), and a stroke (brain attack), i.e. a cerebrovascular accident (CVA).
I. Local (graded potentials) vs action potentials
   A. Local potentials: For short distances information is transmitted via "local" or "graded" potentials.
   B. Action potentials: A specialized signal called an "action potential" is used to transmit information over long distances.
   C. Overview of the two types of electrical signals that the nervous system uses: local (graded) potentials and action potentials.

### Comparison of Graded Potentials and Action Potentials

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Graded Potentials</th>
<th>Action Potentials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin</td>
<td>Arise mainly in dendrites and cell bodies</td>
<td>Arise at trigger zones and propagate along axon</td>
</tr>
<tr>
<td>Types of channels</td>
<td>Chemical, mechanical, or light</td>
<td>Voltage gated ion channels</td>
</tr>
<tr>
<td>Conduction</td>
<td>Not propagated, localized, thus permit communication over a few mm</td>
<td>Propagated, thus permit communication over long distances</td>
</tr>
<tr>
<td>Amplitude</td>
<td>Depends on strength of stimulus; varies from less than 1 mV to more than 50mV</td>
<td>All or none, typically 100 mV</td>
</tr>
<tr>
<td>Duration</td>
<td>Typically longer, ranging from ms to several minutes</td>
<td>Typically shorter, ranging from 0.5 - 2 ms (milliseconds)</td>
</tr>
<tr>
<td>Polarity</td>
<td>May be hyperpolarizing (inhibitory to generation of action potential) or depolarizing (excitatory to generation of action potential)</td>
<td>Always consist of depolarizing phase followed by repolarizing phase and then return to resting membrane potential</td>
</tr>
<tr>
<td>Refractory Period</td>
<td>No, thus exhibit temporal and spatial summation</td>
<td>Yes, therefore not subject to summation</td>
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</table>

1. Major characteristics of local potentials: they are graded in amplitude with the stimulus, and they die out (decrement) with distance. Local potentials include receptor, synaptic, motor endplates, and pacemaker potentials. Local potential decrease as the currents spread spatially because ions leak through the membrane causing a loss of charge.
2. Major characteristics of action potentials: they are large, brief, invariant (in amplitude) signals that propagate along axons without decrement. They are called "all or none" because once they are initiated, their amplitude is independent of the stimulus that caused them. Action potentials are produced primarily by voltage-gated Na\(^+\) and K\(^+\) ion channels. (In heart muscle and smooth muscle, Ca\(^{2+}\) also is involved in the depolarizing portion of the action potential). Precise timing in opening and closing of channels gives transient depolarizations in the membrane potential that can travel down the axon at speeds up to 120 meters per second.

II. Ionic basis of action potentials

Cells with excitable membranes produce action potentials - nerves, muscles, some glandular cells. Fig. 5 shows the dominant features of the action potential: a large spike that lasts about one msec (called overshoot when membrane is positive), an undershoot below the resting potential, and recovery. What makes these cells able to produce spikes? The answer is the presence in the membrane of **voltage-gated** sodium channels (Na\(_v\)s).

A. Hodgkin and Huxley's (H&H) Nobel-prize-winning Na\(^+\) hypothesis for action potential production
1. The large positive spike of the action potential is due to an increase in permeability (conductance) to Na\(^+\). During the action potential, Na\(^+\) rushes into the cell, driving the cell away from its negative resting potential which is near \(E_{K^+} (-90 \text{ mV})\), in an upstroke toward \(E_{Na^+} (+60 \text{ mV})\). A later outward flow of K\(^+\) ions causes an undershoot (hyperpolarizing afterpotential) of the action potential. H\&H studied the action potential in squid giant axons which are big and easy to study, but you can apply these findings directly to retinal ganglion cells whose axons form the optic nerve of the eye. Figure 6 shows the increases in conductance (g) for Na\(^+\) and for K\(^+\) ions during an action potential (marked \(E_m\)).

2. Sea-water experiments of H and H (Fig. 7).

   a) By clamping (holding) the membrane at set voltages and measuring current flow (by ions), when either Na\(^+\) or K\(^+\) alone was present in the bath, they observed the inward Na\(^+\) current (depolarization), and the outward K\(^+\) current (hyperpolarizing afterpotential). These experiments allowed H\&H to study the time course of the changes in conductance (gNa\(^+\) and gK\(^+\)) during the action potential: the conductance could be calculated using Ohm's Law, \(E=IR\), and transposing, \(R=E/I\) [\(E\) is the voltage where the membrane was clamped, \(I\) is the current that they recorded (flow of ions). They solved for \(R\), the resistance]. Conductance (g) is the inverse of resistance. The conductance was later measured for single ion channels using patch clamp recording techniques in the nerve cells of many different animals.
b. In more modern experiments, patch electrodes are used to study transport proteins and membrane permeability. Recordings can be made from a small region of membrane or from the whole cell. For the classical experiments in squid, recordings were made by an electrode inside the axon, referenced to an electrode outside.

III. Stages of the action potential (3 different types of channels are involved) (Fig. 8)

A. Depolarization from the resting potential to the threshold for opening voltage-gated Na\(^+\) channels. This occurs because a chemical neurotransmitter opens "ligand-gated" Na\(^+\) channels in the cell membrane and positive charges enter the cell.

B. Opening of voltage-activated Na\(^+\) channels. After the ligand-gated Na\(^+\) channels open, the membrane depolarizes because of the additional sodium that enters. When the membrane has depolarized by about 15 mV, the threshold for the voltage-gated Na\(^+\) channels is reached and they open, which further depolarizes the membrane, leading to opening of more voltage-gated channels (positive feedback) and an action potential occurs due to the rapid influx of Na\(^+\) (i.e., current). The membrane potential approaches \(E_{Na}^+\). Action potentials can be blocked with substances that block the voltage-gated Na\(^+\) channels, e.g. tetrodotoxin (TTX), from the poisonous puffer fish. Other drugs that block these channels include local anesthetics such as lidocaine, =Novocain and cocaine which stop transmission of the pain message via pain fibers (axons) to the brain.
C. Inactivation of voltage-gated Na\(^+\) channels - leads to repolarization of the membrane. During inactivation, action potentials cannot occur; this is called the absolute refractory period.

D. Delayed opening of voltage-gated K\(^+\) channels produces an outward K\(^+\) current also leads to repolarization of the membrane toward \(E_{K^+}\) - undershooting the resting potential. (Relative refractory period).

(If the membrane is continuously depolarized, sodium channels may be inactivated and action potentials will not occur: this is called accommodation.)

Fig. 8 – action potential

Response of squid axon to depolarizing current
When the cell is depolarized to threshold, it fires an action potential.

IV. Propagation of the action potential (Fig. 9)

Fig. 9.
A. Local circuit theory - the next patch of membrane must be depolarized to threshold for the NaV channels in order for the action potential to be propagated in the direction of the patch. Due to the refractory periods, the propagation moves in one direction. To understand the limitations of this propagation, the electrical characteristics of the membrane will be considered:

1. Space and time constants - Local potentials produce current flow down the membrane. Ions leak through the membrane as the local current flows. The length constant tells us the distance that the current flows before it decays to about 1/3 (1/e) of its original value. The space constant is inversely related to the leak of ions: and in electrical terms, less leak is indicative of higher membrane resistance. Thus the space constant increases if membrane resistance increases. Internal resistance is inversely proportional to cross sectional area of the axon. Larger diameter axons will have lower internal resistance, which will speed up axonal conduction velocity.

Equation for the length constant (lambda) (= is better stated, related):

\[ \lambda = \sqrt{\frac{r_m}{r_i}} \]

where:
- \( r_m = \) membrane resistance
- \( r_i = \) internal resistance

Equation for the time constant (tau):

\[ \tau = r_m \times c_m \]

where:
- \( c_m = \) membrane capacitance

The time constant of the membrane tells us how long the membrane takes to charge up, and how long for that charge to decay. In electrical terms, it is directly related to the capacitance of the membrane (ability to hold charge). If the membrane capacitance increases, the time constant increases. A long (slow) time constant means that the potential reaches its maximum level slowly. If, at the same time, the space constant is small (short), the potential may die out before it can propagate the action potential by bringing the next patch of membrane to threshold for the voltage-activated Na⁺ channels. A solution to this problem is found with the myelin sheath. Myelination leads to increased resistance where it is present, thus increasing the length constant. At breaks in myelin, resistance is lower (conductance is higher), and tau is shortened, so the membrane charges up quickly.

**Figure 3-9** An axon or a muscle fiber resembles an electrical cable. Currents (i) that flow across the membrane resistance (\( r_m \)) are lost from the cable. Currents that flow through the longitudinal resistance (\( r_l \)) carry the electrical signal along the cable. The larger the ratio \( r_m/r_l \), the more efficient the signal transmission along the fiber.
Fig. 10. Increasing the membrane resistance ($r_m$) leads to more efficient signal transmission, i.e. less loss of signal.

B. Myelinated axons (Fig. 11 and Fig. 12)

Myelin sheath is a fatty coating of the axons produced in the PNS by Schwann cells, and in the CNS by oligodendrocytes which are a kind of glia. (Glia are not neurons, they have supportive roles in the nervous system.)

1. Myelin sheath speeds axonal conduction velocity: it increases membrane resistance and decreases membrane capacitance.

2. Nodes of Ranvier - breaks in the myelin lead to saltatory conduction - the potential “jumps” from node to node where the voltage gated channels are congregated.

3. Saltatory conduction is assisted by uneven distribution of voltage-gated Na$^+$ and K$^+$ channels. Density of voltage-gated Na$^+$ channels (per sq micrometer) – these are flanked by K$^+$ channels.

   - Nodes of Ranvier: 2000-12000
   - Initial segment: 350-500
   - Under myelin: <25
   - Axons of unmyelinated nerves: 110
   - Cell body: 50-75
   - Axon terminals: 20-75

4. There are demyelinating diseases such as the auto-immune disease, multiple sclerosis, where myelin is lost and then returns: the loss of myelin causes signal transmission along the axon to slow down. The normal regional distribution of voltage-gated sodium and potassium channels also is disrupted by changes in myelin.

Fig. 11 Myelin sheath
Large diameter axons conduct faster than small ones, regardless of the myelin (Fig. 12).
For example: 0.5 meters/sec for small, 175 m/sec for large myelinated axons: time from head to
toe would be 4 sec vs 0.01 sec.

Fig. 12 Conduction velocity of myelinated and unmyelinated axons
D. Local potentials and action potentials in the retina

Different types of retinal neurons use the different kinds of potentials to transmit electrical signals to the next cell.

1. Overview of the retina. (Fig. 13)
   a. Photoreceptor cells that transduce light energy to neural signals
   b. Bipolar cells process and relay signals to retinal ganglion cells
   c. Horizontal and amacrine cells make lateral connections – mostly local potentials
   d. Retinal ganglion cells whose axons form the optic nerve (cranial nerve II) that send visual signal from the eye to the brain.

2. Neural signaling by retinal cells.
   a. Photoreceptors, bipolar, and horizontal cells are very close together (i.e. they have short axons), and they produce local graded potentials.
   b. Retinal ganglion cells which send the signal over a long distance from the eye to the brain, communicate via action potentials.
   c. Photoreceptors produce a special kind of local potential, a "receptor potential" when they transduce (change) light energy to a neural signal. The receptor cells in
other sensory systems also produce receptor potentials. In the auditory and vestibular systems, the receptor potentials are produced by hair cells in the cochlea of the ear; in the case of touch there are a number of receptors, such as those that are pressure or temperature sensitive.

d. Resting potentials for the different types of retinal cells vary. For instance, photoreceptors rest around -40 mV and they hyperpolarize when they transduce light signals. In contrast, ganglion cells which depolarize and produce spikes, may rest around -70 mV.
Lecture 4. Nerve fibers; Synaptic transmission I

I. Size and speed of axons in various portions of the peripheral nervous system (all myelinated by Schwann cells (Tables 2-1-2.3; Fig. 13: all from Ganong, 19th -21st editions)

A. Speed of conduction and relationship to axon diameter: The fastest fibers both in conduction velocity and spike duration are large myelinated "A" fibers, "B" fibers are smaller, slower myelinated fibers, and "C" fibers are small very slow unmyelinated fibers (Table 2-1). When the fibers are sensory, a numerical classification is sometimes used (Table 2-2)

B. Blocking axonal conduction - by cold, anoxia, compression, and drugs. Cold - large fibers blocked at 7 deg. C, small at 3 deg C. Local anesthesia affects small C pain fibers before larger fibers. (Tables below, 2.1-2.3)

C. The compound action potential - the extracellularly recorded potential generated by a nerve made up of many fibers (axons) of different caliber. Because of the different fiber types, the compound action potential can display a number of response peaks. The fastest conducting fibers produce the first recorded peak after a stimulus, as in Fig. 14, and the slower peaks are more separated as distance from the stimulator increases.

---

![Fig. 14](image)

**Table 2-1. Nerve fiber types in mammalian nerve.**

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>Function</th>
<th>Fiber Diameter (μm)</th>
<th>Conduction Velocity (ms)</th>
<th>Spike Duration (ms)</th>
<th>Absolute Refractory Period (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Proprioception; somatic motor</td>
<td>12-20</td>
<td>70-120</td>
<td>0.4-0.5</td>
<td>0.4-1</td>
</tr>
<tr>
<td>B</td>
<td>Touch, pressure</td>
<td>5-12</td>
<td>30-70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>γ</td>
<td>Motor to muscle spindles</td>
<td>3-6</td>
<td>15-30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ</td>
<td>Pain, cold touch</td>
<td>2-5</td>
<td>12-30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Preganglionic autonomic</td>
<td>&lt;3</td>
<td>3-15</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>C</td>
<td>Dorsal root</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sympathetic</td>
<td>Postganglionic sympathetic</td>
<td>0.3-1.3</td>
<td>0.7-2.3</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

**Table 2-3. Relative susceptibility of mammalian A, B, and C nerve fibers to conduction block produced by various agents.**

<table>
<thead>
<tr>
<th>Susceptibility to:</th>
<th>Most Susceptible</th>
<th>Intermediate</th>
<th>Least Susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxia</td>
<td>B</td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td>Pressure</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Local anesthetics</td>
<td>C</td>
<td>B</td>
<td>A</td>
</tr>
</tbody>
</table>

1 A and B fibers are myelinated; C fibers are unmyelinated.
II. Retinal ganglion cell axons

The axons of retinal ganglion cells carry information from the retina to the brain via action potentials (Fig. 15):

<table>
<thead>
<tr>
<th>Number</th>
<th>Origin</th>
<th>Fiber Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>Muscle spindle, annulo-spiral ending.</td>
<td>A α</td>
</tr>
<tr>
<td>lb</td>
<td>Golgi tendon organ.</td>
<td>A α</td>
</tr>
<tr>
<td>II</td>
<td>Muscle spindle, flower-spray ending; touch, pressure.</td>
<td>A β</td>
</tr>
<tr>
<td>III</td>
<td>Pain and cold receptors; some touch receptors.</td>
<td>A δ</td>
</tr>
<tr>
<td>IV</td>
<td>Pain, temperature, and other receptors.</td>
<td>Dorsal root C</td>
</tr>
</tbody>
</table>

A. Diameters of optic tract fibers (axons) are in three main groups: between 0.2 and 1.8 microns.

B. Myelination of retinal ganglion cell axons: the axons are not myelinated in the retina. They travel to the optic nerve head where they exit the eye; then they acquire myelin. The fibers in the optic nerve are myelinated like CNS axons, i.e. myelin is produced by oligodendrocytes in the CNS. (Schwann cells produce the myelin sheath in the peripheral nervous system, e.g. around motor nerves that leave the spinal cord.) Thus the optic nerve (prechiasm) and optic tract (post chiasm) are tracts of the CNS. The retina has been studied extensively as an "approachable part of the brain".
C. Axonal conduction velocity: Slow and fast axons in the optic nerve have conduction velocities ranging from less than 10 meters/sec (m/s) for some slow axons, to more than 30 m/s for fast axons. At 25 m/s information can travel the ~8 cm from the retina to the lateral geniculate nucleus (LGN) in the thalamus in 3.2 msec. These differences in axonal conduction velocity in the optic nerve have important implications for vision. The fast and slow pathways remain separate as they project in parallel to the LGN and then to the primary visual cortex.

III. Synapses - 2 types:

A. Electrical - gap junctions - ions pass from cell to cell, and the electrical potential spreads – seen, for example in horizontal connections in the retina (horizontal cells and amacrine cells). These junctions are made up of connexons, composed of 6 subunits. (Fig. 16). They can be in open or closed states, due to neurotransmitters/modulator-stimulated release of 2nd messengers. When they are closed, molecules do not pass from cell to cell.

B-1. Chemical (classical) - a neurotransmitter is released from clear vesicles at the active zone to pass the neural signal the next cell. The chemical signal changes the transmembrane potential of the next cell by causing ion channels to open, leading to either depolarization or hyperpolarization of the membrane of the postsynaptic cell, depending on the transmitter and the ion channels that affected. CNS: glutamate (Excitatory), GABA and Glycine (inhibitory), catecholamines (dopamine, norepinephrine, epinephrine), acetylcholine, etc (see Table at end of handout).

PNS: acetylcholine, catecholamines.

B-2: Transmission via neuromodulators released from dense core vesicles. Neuromodulators generally bind to receptors in the postsynaptic cell membrane triggering a signal-transduction cascade (several steps) in the cell that modifies cellular activity. These may also be neurotransmitters, or other peptides etc.
Fig. 17A Axo-dendritic synapse in the CNS -

Fig. 17B Axo-dendritic synapse in the CNS – Note the presence of voltage-gated Ca\textsuperscript{2+} channels

Fig. 18
1. Structure of the chemical synapse (Figs. 18 – 19)
   a. Pre-synaptic membrane (active zone) - where release of transmitter occurs. Note that there are voltage-gated calcium channels in the active zone of the pre-synaptic membrane. Opening of these channels is essential for transmitter release to occur.
   b. Post-synaptic membrane - on the recipient cell — dendrite in Fig. 17A, but it could be a soma or another axon. Transmission is one-way — pre to post
   c. Synaptic cleft - space between the pre- and post-synaptic membrane.

   The chemical synapse is very important for transmission of signals. It also is an important site due to its susceptibility to manipulation and change: plasticity associated with learning, accessibility to toxins, locus of pathological conditions such as myasthenia gravis. The synapse’s sensitivity to ion changes and pharmacological agents provides a location for pharmacologically manipulating the nervous system.

2. Synaptic delay: delay between arrival of the action potential in the pre-synaptic terminal and the membrane potential changes in the post-synaptic cell — for < 0.5 to 1 msec in the CNS.

   C. Example of a well-studied chemical synapse: The neuromuscular junction (NMJ) of the PNS (Fig. 19). The region called the motor endplate includes: the pre-junctional membrane, the post-junctional membrane, and the cleft. Synaptic transmission was studied first at the neuromuscular junction because it is more accessible to study than the synapses in the CNS where neurons are densely packed. The neurotransmitter released from the motor neuron axon at the NMJ is acetylcholine (ACH).
IV. Criteria for a substance to qualify as a neurotransmitter.

*ACH in the NMJ has met these criteria*

1. Present pre-synaptically
2. Released by physiological stimulus in sufficient amounts
3. Identical action to the endogenous transmitter
4. Mechanisms to terminate the transmitter action rapidly must exist

V. Events in the pre-synaptic terminal that lead up to transmitter release in the active zones

A. Depolarization - due to an action potential (or local potential in some cases, e.g. bipolar cells in the retina) in the presynaptic cell

B. Entry of Ca$^{2+}$ into the presynaptic terminal via voltage-gated Ca$^{2+}$ channels

C. Transmitter release - requires Ca$^{2+}$.
   1. Ca$^{2+}$ causes the vesicles to fuse with the membrane and release the transmitter into the cleft by exocytosis.
   2. Vesicular release of transmitter

Fig. 20: The process of exocytosis in classical neurotransmitter release. Note the critical role of calcium in fusion/exocytosis. Note also that the vesicles are recycled via endocytosis, translocation, endosome fusion and budding

Quantal nature of transmitter release - The release is in packets of molecules, not solitary molecules. The smallest electrical response of NMJ post-synaptic membrane is due to release of the contents (i.e. all the molecules of ACH) in one vesicle. Quantal release was first described for the NMJ, but the general principles apply for most synaptic transmission. Small (0.5 mV) spontaneous post-synaptic potentials were observed in the postsynaptic muscle, and were called miniature endplate potentials (MEPPS). Delivery of about 5000 molecules of ACH to the muscle produced one MEPP, i.e. the muscle fiber's response to release from one vesicle. In neurons in the CNS, these are called “Mini post synaptic potentials”, Minis, either excitatory
(E) or inhibitory (I). Much recent research in the CNS has focused on establishing mechanisms of vesicular release, and the size of quanta.

Fig. 21
SNARE (SNAP [soluble NSF (N-ethylmaleimide-sensitive fusion protein) attachment protein] receptor) proteins that are involved in synaptic vesicle docking and fusing are shown in this figure. Another name for Synaptobrevin is VAMP (vesicle associated membrane protein).

These SNARES are the target of bacterial neurotoxins: Botulinum toxin and Tetanus toxin. The interactions of different forms of Botulinum toxin (BoNT or Botox) with various snares are illustrated in the figure below. The interaction leads to reduction of neurotransmission. This substance can be injected locally to reduce release of acetylcholine onto skeletal muscles, which can relax the muscles.
Post - synaptic transmission

I. The post-synaptic membrane [illustrated by the neuromuscular junction (NMJ) in Fig. 19, but similar to CNS neuron to neuron synapses, but with a variety of different neurotransmitters]

A. There are acetylcholine (ACH) receptors on the post-junctional membrane of the motor endplate on skeletal muscle.

B. Binding of ACH to its receptor leads to conformational changes that open cation, e.g. Na⁺, channels in the post-junctional membrane. Patch clamp studies of single Na⁺ channels have shown that the channels are open for about 1 millisecond. The opening of the cation channels leads to excitatory postsynaptic potentials (EPSPs, or EPPs) in the motor endplate.

C. The action of ACH at the receptor is brief because ACH is quickly cleared from the synaptic cleft in 3 basic ways:

1. Degradation (enzymatic destruction): ACH is broken down by the enzyme acetylcholinesterase (ACHE) in the cleft to choline and acetate.
2. Diffusion: ACH diffuses away (in some cases)
3. Reuptake (active transport): The choline from ACH is taken back up by the pre-junctional (synaptic) membrane. See Fig. 22 below

Fig. 22
II. Receptors for neurotransmitters, that are involved in signaling, and neuromodulators that alter the state of the cell.

Generally there are several types of receptors for each neurotransmitter, and it is the particular receptor types that determine the specificity of the neurotransmitter action.

A. Receptor agonists: substances that mimic the action of the neurotransmitter.

Receptors are classified by their agonists.

Examples: Nicotinic ACH receptors at the NMJ bind nicotine (an agonist) exogenously applied, and it has the same action as ACH released from the neuron.

Muscarinic ACH receptors on smooth muscles of the iris (pupil sphincter muscle) bind the agonist muscarine exogenously applied and it has the same action as ACH.

Also see glutamate receptor types below

B. Receptor antagonists: substances that block the action of the neurotransmitter at the post-synaptic membrane.

Examples: Curare blocks nicotinic receptors
Atropine or tropicamide block muscarinic receptors

C. Neurotransmitters and second messengers: Neurotransmitters bind to receptors, and affect ion channels either directly, or via second messengers (Fig. 24). When transmitters affect ion channels directly, the receptors are ionotropic, also called "channel-linked" and they mediate fast transmission (e.g. nicotinic receptors are ionotropic). Neurotransmitters can also affect
channels via intracellular signal pathways involving G-proteins, that lead to increases or decreases in concentrations of second messengers such as Ca\(^{2+}\), or cyclic AMP, cyclic GMP. These receptors are metabotropic or "non-channel linked", (e.g. muscarinic receptors are metabotropic) and they mediate slow transmission. G-protein coupled receptors (GPCRs) and second messengers will be discussed in the next lecture.

Fig. 24 Examples of ionotropic and metabotropic receptors

D. Excitatory neurotransmitters

1. In the PNS, acetylcholine (ACH) is the excitatory transmitter released by motor neurons making synapse on skeletal muscle (NMJ). The ACH receptors on the muscle are nicotinic receptors because nicotine is an agonist that mimics the action of ACH at these receptors. The nicotinic ACH receptor can be blocked by antagonists curare, and alpha neurotoxins such as \(\alpha\)-bungarotoxin, and cobrotoxin which are small proteins from snake venom. ACH also is the transmitter for innervation of some smooth (automomic NS) and cardiac muscles; the receptors are muscarinic receptors (M1, M2 etc.) because the toxin, muscarine has the same action as ACH at these receptors. They are blocked by atropine. Norepinephrine is the transmitter for innervation of other smooth muscles via adrenergic alpha or beta receptors.

2. In the CNS, including the retina, the major excitatory transmitter is the excitatory amino acid (EEA), glutamate (aspartate in some invertebrates). There are several types of glutamate glutamate (see figures). ACH and the biogenic amines such as norepinephrine also can be excitatory transmitters in the CNS.
E. Inhibitory neurotransmitters and their receptors

In the PNS and CNS, including retina, **GABA and glycine, both amino acids, are the major inhibitory neurotransmitters**. Inhibitory neurotransmitters cause IPSP’s in the postsynaptic cell. There are ionotropic and metabotropic subtypes of inhibitory receptors. GABA<sub>A</sub> or C receptors and glycine receptors are ionotropic. Binding of the ligand increases gCl<sup>-</sup>; GABA<sub>B</sub> receptors are metabotropic; binding of GABA increases gK<sup>+</sup> (Fig. 23), and gCa<sup>2+</sup> decreases.
Fig. 27 Ionotropic (left) and metabotropic GABA receptors (right) (Ganong, 23rd edition)

Major neurotransmitters in the retina

Neurotransmitters in the retina

Glutamate

GABA
Glycine
ACH
Dopamine
Etc.
III. Post-synaptic (or post-junctional) potentials (PSPs). - local, graded potentials.

Fig. 28: Interaction of EPSP’s and IPSPs at the postsynaptic neuron.

A. Excitatory (EPSPs) are depolarizing: the neurotransmitter (an excitatory transmitter, e.g. glutamate) opens sodium channels and gNa+ is increased relative to g K+. For example, in skeletal muscle the ratio of gNa+ to gK+ changes to about 1.25 as the potential moves upward. The response decays in space and time due to the membrane resistance and capacitance. If the threshold for an action potential occurs, the signal can be transmitted over long distances.

B. Inhibitory (IPSPs) are hyperpolarizing or membrane stabilizing: the neurotransmitter (an inhibitory transmitter) opens chloride or potassium channels, i.e. gCl⁻ or gK⁺ are increased. If gK⁺ alone is increased then the membrane potential moves toward E_K⁺, which is more hyperpolarized than the resting potential. If gCl⁻ is increased, and the membrane is already depolarized, then a hyperpolarization will occur. However, if the membrane is sitting near the equilibrium potential for chloride, the potential may not change, but since there is increased gCl⁻, the size of EPSP's occurring at the same time will be reduced (short-circuited or shunted) because depolarization due to the entry of Na⁺ into the cell will be balanced by the hyperpolarization due to entry of Cl⁻.

C. Spatial and temporal summation of PSP's allows neurons to integrate information from many inputs. (Fig. 24)

1. **Spatial summation:** Post synaptic potentials due to various inputs from other neurons occurring at different points on the membrane can sum spatially in the post-synaptic neuron. The membrane may then reach threshold for an action potential. The distance over which spatial summation can occur is determined by the space constant.

2. **Temporal summation:** Potentials from the same inputs occurring at different times can sum temporally in the post-synaptic neuron. The membrane may then reach threshold for an action potential. The time over which summation occurs is determined by the time constant.
Table 3: Selected neurotransmitters

<table>
<thead>
<tr>
<th>Category</th>
<th>Neurotransmitter</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acetylcholine</strong></td>
<td><em>Acetyl Choline</em> (motor neurons)</td>
<td>m/i</td>
</tr>
<tr>
<td></td>
<td><em>Serotonin</em> (memory, emotion)</td>
<td>m/i</td>
</tr>
<tr>
<td><strong>Monoamines</strong></td>
<td><em>Dopamine</em> (motivation, addiction)</td>
<td>m</td>
</tr>
<tr>
<td></td>
<td><em>Norepinephrine / Noradrenaline</em> (sympathetic NS)</td>
<td>m</td>
</tr>
<tr>
<td></td>
<td><em>Epinephrine / Adrenaline</em> (sympathetic NS)</td>
<td>m</td>
</tr>
<tr>
<td><strong>Amino Acids</strong></td>
<td><em>Glutamate</em> (most prominent in CNS, excitatory)</td>
<td>m/i</td>
</tr>
<tr>
<td></td>
<td><em>GABA</em> (30-40% of CNS synapses, inhibitory)</td>
<td>m/i</td>
</tr>
<tr>
<td></td>
<td>Aspartate</td>
<td>l</td>
</tr>
<tr>
<td></td>
<td>Glycine*</td>
<td>i</td>
</tr>
<tr>
<td></td>
<td>Adenosine</td>
<td>m</td>
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<tr>
<td></td>
<td>Guanosine</td>
<td>m</td>
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<tr>
<td></td>
<td>ATP</td>
<td>l/m</td>
</tr>
<tr>
<td></td>
<td>Others</td>
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<tr>
<td><strong>Purines</strong></td>
<td>Endorphins</td>
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<tr>
<td><strong>Neuropeptides</strong></td>
<td><em>Enkephalins</em></td>
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</tr>
<tr>
<td></td>
<td><em>Dynorphins</em></td>
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<tr>
<td></td>
<td>Substance P</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neuropeptide Y</td>
<td>m</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td></td>
</tr>
<tr>
<td><strong>Soluble Gases</strong></td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CO</td>
<td></td>
</tr>
</tbody>
</table>

* can be excit. or inhib., depending on Cl⁻ conc.
Lecture 5. RECEPTORS in the nervous system: Ligand-gated ion channels (LGICs), G-protein-coupled receptors (GPCR) and signaling cascades

I. Receptors

A receptor is specific protein in either the plasma membrane or the interior of a target cell to which a chemical mediator (ligand) binds to exert its effects.

Review of major types of receptors

1. **Ionotropic**, channel-linked, i.e. a ligand gated ion channel (LGIC) - The neurotransmitter binds to the receptor protein, which opens the ion channel.
2. **Metabotropic**, non-channel linked – i.e. G-protein coupled receptor (GPCR).
   a. Ion channels are opened via actions of second messengers
   b. Other cellular events also are mediated by second messengers.
3. **Protein tyrosine kinases (RTKs)**
   a. Receptor for the hormone insulin, and some growth factors
4. **Nuclear hormone receptors**
5. Receptors located in cytosol for some steroids and other compounds

Table 1.

<table>
<thead>
<tr>
<th>Type</th>
<th>Subtype</th>
<th>Endogenous Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LGICs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>Nicotinic</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>GABA</td>
<td></td>
<td>GABA</td>
</tr>
<tr>
<td>Glutamate</td>
<td>NMDA, kainate, AMPA</td>
<td>Glutamate or aspartate</td>
</tr>
<tr>
<td>Serotonin</td>
<td>5-HT3</td>
<td>Serotonin.</td>
</tr>
<tr>
<td><strong>GPCRs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACTH</td>
<td></td>
<td>ACTH</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>Muscarinic</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>Adrenergic</td>
<td>α1, β1-2, β1-3</td>
<td>Epinephrine and NE</td>
</tr>
<tr>
<td>GABA</td>
<td>B</td>
<td>GABA</td>
</tr>
<tr>
<td>Glucagon</td>
<td></td>
<td>Glucagon</td>
</tr>
<tr>
<td>Glutamate</td>
<td>Metabotropic</td>
<td>Glutamate</td>
</tr>
<tr>
<td>Opioid</td>
<td>µ, κ, δ</td>
<td>Enkephalins</td>
</tr>
<tr>
<td>Serotonin</td>
<td>5-HT1,2,4,5,7</td>
<td>5-HT</td>
</tr>
</tbody>
</table>

**II. The role of G-proteins**

A. G-proteins are GTP (guanosine triphosphate) regulatory proteins that serve as molecular switches that regulate intracellular processes. G-proteins exist between “On” and “Off” states.

1. Heterotrimeric G-proteins are composed of three subunits: alpha, beta, and gamma. (Fig. 1)

Fig. 1 (Ganong 23rd edition, 2-23)
B. Over 100 different receptors communicate through 30 known heterotrimeric G-proteins (40-45 kd) (Table 1&2). The G-proteins are involved in signal transduction, i.e. they are intermediaries for membrane receptors that bind extracellular ligands that control numerous intracellular processes.

C. Other classes of small "low molecular weight" monomeric G-proteins (20-35 kd) are involved in intracellular processes such as growth and differentiation etc. (see Table 2).

D. GPCRs initiate responses that are slower and more diverse than channel-linked receptors. They initiate a chain of events called a cascade. G-protein cascades regulate ion channels, cause changes in growth, metabolism, cytoskeletal structure, and gene expression.

E. GPCRs are membrane bound proteins, such as rhodopsin in photoreceptor outer segments, muscarinic receptors for ACH, or adrenergic receptors for norepinephrine (NE) AKA noradrenaline (NAdr), Epinephrine E AKA Adr, Dopamine (DA) etc., which activate the G-protein alpha subunit of the associated G-protein.

F. The effects of G-proteins generally occur through changes in concentration of intracellular signaling molecules called second messengers.

G. G-protein cascades generally involve 3 or more steps. This allows amplification (increasing the signal) at each step (Fig. 2, 3 and Table 2).

Fig. 2 and Table 2

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Receptor</th>
<th>G protein</th>
<th>Effector</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. photon</td>
<td>rhodopsin</td>
<td>$G_T$</td>
<td>PDE</td>
</tr>
<tr>
<td>2. odorant</td>
<td>olfactory</td>
<td>$G_{olf}$</td>
<td>AC</td>
</tr>
<tr>
<td>3. NAdr</td>
<td>$\beta$-adrenergic</td>
<td>$G_S$</td>
<td>AC</td>
</tr>
<tr>
<td>4. ACh</td>
<td>$M_2$-muscarinic</td>
<td>$G_K$</td>
<td>$K^+$ channel</td>
</tr>
<tr>
<td>5. vasopressin</td>
<td>V-1a</td>
<td>$G_{PLC}$</td>
<td>PLC</td>
</tr>
<tr>
<td>6. glutamate</td>
<td>mGluR6</td>
<td>Go</td>
<td>TRP/ cation channel</td>
</tr>
</tbody>
</table>

On bipolar cells

TRP = transient receptor potential
III. Basic elements of the G-protein signaling pathway

A. Extracellular messenger binds to a receptor, which activates a G-protein. This means that it binds to GTP. In its inactive form, it is bound to GDP.

B. G-proteins activate effectors - ion channels, or enzymes, that regulate second messengers.

C. The second messengers may activate protein kinases.

D. Protein kinases phosphorylate proteins. When a protein is phosphorylated its activity may be enhanced or suppressed. Selective phosphorylation of different amino acids in intracellular signaling sequences is an important theme that recurs throughout the neuro-regulatory pathways. Phosphorylation can lead to opening of ion channels or other physiological effects.

E. Protein phosphatases reverse effects of phosphorylation. At a given time the amount of a given regulated protein is phosphorylated is the results of a balance between the action of kinases and phosphatases on that protein.
IV. Second messengers

Second messengers are components of molecular “circuits” inside cells that regulate metabolism, synthetic activity, and other behavior of the cell. Some major second messengers are:

A. Cyclic adenosine monophosphate (cAMP) (Fig. 4 & 5) The designation of “second messenger” was first given to cAMP. (cyclic AMP)

B. Cyclic guanosine triphosphate (cGMP) (e.g. phototransduction cascade, Fig. 6)

C. Ca^{2+} in complex with the protein, calmodulin - activates dedicated or multifunctional protein kinases that phosphorylate proteins e.g. regulation of the availability of synaptic vesicles.

D. Diacylglycerol (DAG) (or diglyceride) and Inositol Triphosphate (IP3) (Fig. 5)

E. Other small molecules, e.g. Nitric Oxide and arachidonic acid metabolites

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**Fig. 4**

<table>
<thead>
<tr>
<th>Beta adrenergic E effectiveness &gt; NE</th>
<th>Alpha 2 Adrenergic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine 1,3</td>
<td>Dopamine 2, 4</td>
</tr>
</tbody>
</table>

Basic cascade configuration for adrenergic receptors beta and alpha-2

Left – beta and right, alpha-2

Ganong Fig 1-42 The cAMP system. Activation of adenylyl cyclase (AC) catalyzes the conversion of ATP to cAMP. Cyclic AMP activates protein kinase A, which phosphorylates proteins, producing physiologic effects. Stimulatory ligands bind to stimulatory receptors and activate AC via Gs. Inhibitory ligands inhibit AC e via inhibitory receptors.
Fig. 5 Cascade following activation of adrenergic alpha-I receptors (example: sympathetic stimulated by phenylephrine, an agonist for NE that will dilate the pupil)

Fig. 5 Ganong Release of inositol triphosphate (IP$_3$) and diacylglycerol (DAG) as second messengers. Binding of ligand to G protein-coupled receptor activates phospholipase C (PLC $\beta$). Alternatively tyrosine kinase activation of receptors can activate PLC$\gamma$. Hydrolysis of PIP$_2$ occurs, and this produces IP$_3$ which releases Ca$^{2+}$ from the endoplasmic reticulum (ER), and produces DAG which activates protein kinase C (PKC) CaBP. Ca$^{2+}$ binding proteins.

Fig. 6, Summary of adrenergic GPCRs
V. The phototransduction cascade (Fig. 6A,B,C)

A. Photoreceptor structure: Outer segment (OS), inner segment (IS), connected by a modified cilium
   1. Rod OS: cylindrical stack of membranous discs enclosed by surface membrane. 90-95% of protein in disc membranes is rhodopsin, the receptor for quanta.
   2. Rod IS: houses the nucleus, ER, mitochondria; responsible for energy production and protein synthesis

B. The photoreceptor G-protein cascade - end result: light hyperpolarizes rods by closing cGMP-activated cation channels which allow Na⁺ (and Ca²⁺) to flow into the cell when open. Channel closure interrupts the dark current.
   1. When a photon of light is absorbed, rhodopsin in the disc membrane is isomerized (from cis to trans configuration), a transitional intermediate is = R* (or Rh*, activated rhodopsin)
   2. R* catalyzes activation of the G-protein, transducin (exchange bound GDP for GTP). This activates the effector, phosphodiesterase (PDE) by removing gamma inhibitory subunits.
   3. Activated PDE hydrolyzes cGMP. This leads to closure of cGMP-activated cation channel, and the photoreceptor cell hyperpolarizes. (In the dark, cGMP activates (opens) cation channels which let Na⁺ into the cell and keep it depolarized at about -40 mV).
   4. Termination of the response
      a. Phosphorylation of R* (to desensitize) and binding of arrestin to inactivate R*
      b. Hydrolyze GTP to GDP to inactivate the G-protein, transducin.
      c. Inactivate PDE
      c. Resynthesis of cGMP via the enzyme guanylate cyclase (GC). Reopening of cGMP-activated cation channels.

Fig. 7A,B,C: Rod photoreceptor transduction cascade
Fig. 7B

(A) ROD PHOTORECEPTOR

Light-sensitive outer segment

Dark current depolarizing the cell

Light-insensitive inner segment

Synaptic terminal releasing glutamate

(B) VISUAL CASCADE

\[ \text{Rh} \rightarrow \text{G}_T \text{-GDP} \rightarrow \text{G}_T \text{-GTP} \rightarrow \text{Inactive PDE} \rightarrow \text{Active PDE} \]

\[ \text{cGMP} \]

Holds channels open in dark making dark current

GMP

Some channels close in light

The material past this point is not included in exams

Fig. 7C – Boxes enclose proteins with disease-related mutations
VI. There are many diseases associated with G-protein coupled receptors. Some examples are included in the Tables 1 and 2 below from Ann Rev Med (2004) and can also be found in Ganong, 23rd edition, 2010.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Disease</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cone opsins</td>
<td>Color blindness</td>
<td>X-linked; autosomal recessive</td>
</tr>
<tr>
<td>Rhodopsin</td>
<td>Retinitis pigmentosa</td>
<td>Autosomal dominant; recessive</td>
</tr>
<tr>
<td>V2 vasopressin</td>
<td>Nephrogenic diabetes insipidus</td>
<td>X-linked</td>
</tr>
<tr>
<td>ACTH</td>
<td>Familial ACTH resistance</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>LH</td>
<td>Male pseudohermaphroditism</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>Ca(^{2+}) sensing</td>
<td>Familial hypocalciuric hypercalcemia</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>Ca(^{2+}) sensing</td>
<td>Neonatal hyperparathyroidism</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>Endothelin-B</td>
<td>Hirschsprung disease</td>
<td>Complex</td>
</tr>
<tr>
<td>FSH</td>
<td>Hypergonadotropic ovarian failure</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>TSH</td>
<td>Congenital hypothyroidism</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>TRH</td>
<td>Central hypothyroidism</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>GHRH</td>
<td>Growth hormone deficiency</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>GnRH</td>
<td>Central hypogonadism</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>Melanocortin 4</td>
<td>Extreme obesity</td>
<td>Codominant</td>
</tr>
<tr>
<td>PTH/PTHrP</td>
<td>Blomstrand chondrodysplasia</td>
<td>Autosomal recessive</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Disease</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodopsin</td>
<td>Congenital night blindness</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>LH</td>
<td>Familial male precocious puberty</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>LH</td>
<td>Sporadic Leydig cells tumors</td>
<td>Somatic</td>
</tr>
<tr>
<td>TSH</td>
<td>Familial nonautoimmune hyperthyroidism</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>TSH</td>
<td>Sporadic hyperfunctional thyroid adenomas</td>
<td>Somatic</td>
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<tr>
<td>Ca(^{2+}) sensing</td>
<td>Familial hypocalciuric hypercalcemia</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>PTH/PTHrP</td>
<td>Jansen metaphyseal chondrodysplasia</td>
<td>Autosomal dominant</td>
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