ECE 795:

Quantitative Electrophysiology

Notes for Lecture #1

Wednesday, September 13, 2006

1. INTRODUCTION TO EXCITABLE CELLS

Historical perspective:

- Bioelectricity first discovered by Luigi Galvani in 1780s
- Originally termed “animal electricity”
- Galvani thought that a special electrical fluid was prepared by the brain, flowing through the nerve tubes into muscles

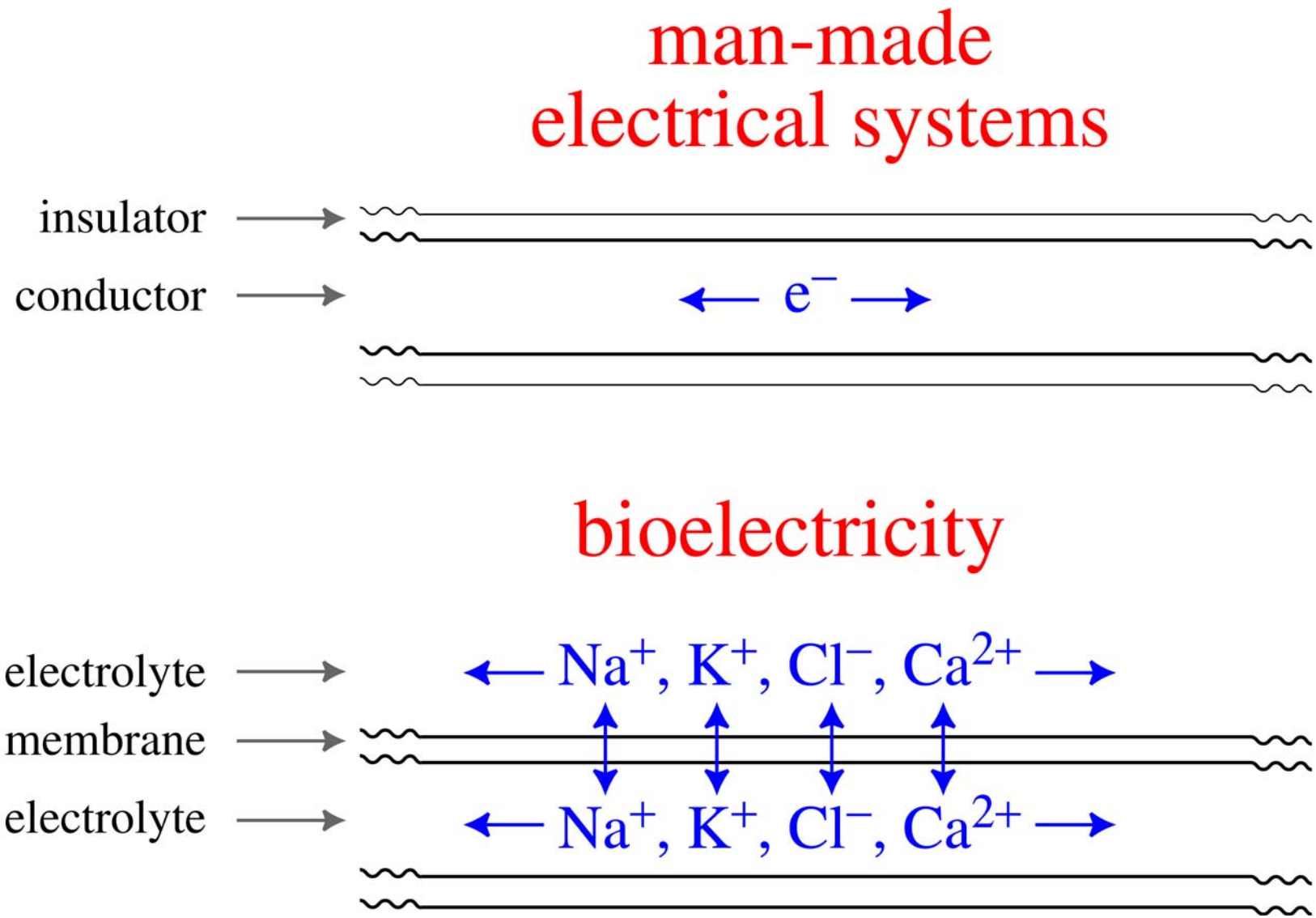
Modern perspective:

- Bioelectricity is now known to obey the same fundamental laws of electricity in the atmosphere, conducting wires, semiconductors, etc.
- However, there are some substantial differences between bioelectrical systems and man-made electrical systems.

Comparison of bioelectricity and man-made electrical systems:

Man-made electrical systems	Charge carriers are electrons within a conductor	Current flow within (insulated) conductors
Bioelectricity	Charge carriers are ions within an electrolyte	Current flow inside *and outside* of (partially-insulated) cell membranes

Comparison (cont.):



Ionic flow in terms of particle movement:

- From the perspective of *chemistry*, ionic movements are described in terms of *moles*, where one mole is:

$$N_A = 6.0225 \times 10^{23} \text{ molecules},$$

  Avogadro's number.

- *Ionic flows* are then described in units of moles per second and *fluxes* (here denoted by the lowercase letter j) in units of moles per second per unit area.

Ionic flow in terms of charge movement:

- From the perspective of *electricity*, ionic movements are described in terms of *columbs*, where an electron (and hence a *univalent ion*) has an electrical charge of 1.6×10^{-19} columbs.
- *Ionic flows* are then described in units of columbs per second (or amperes) and *fluxes* (here denoted by the uppercase letter J) in units of amperes per unit area, e.g., amperes per cm^2.

Converting between particle flow and electrical current:

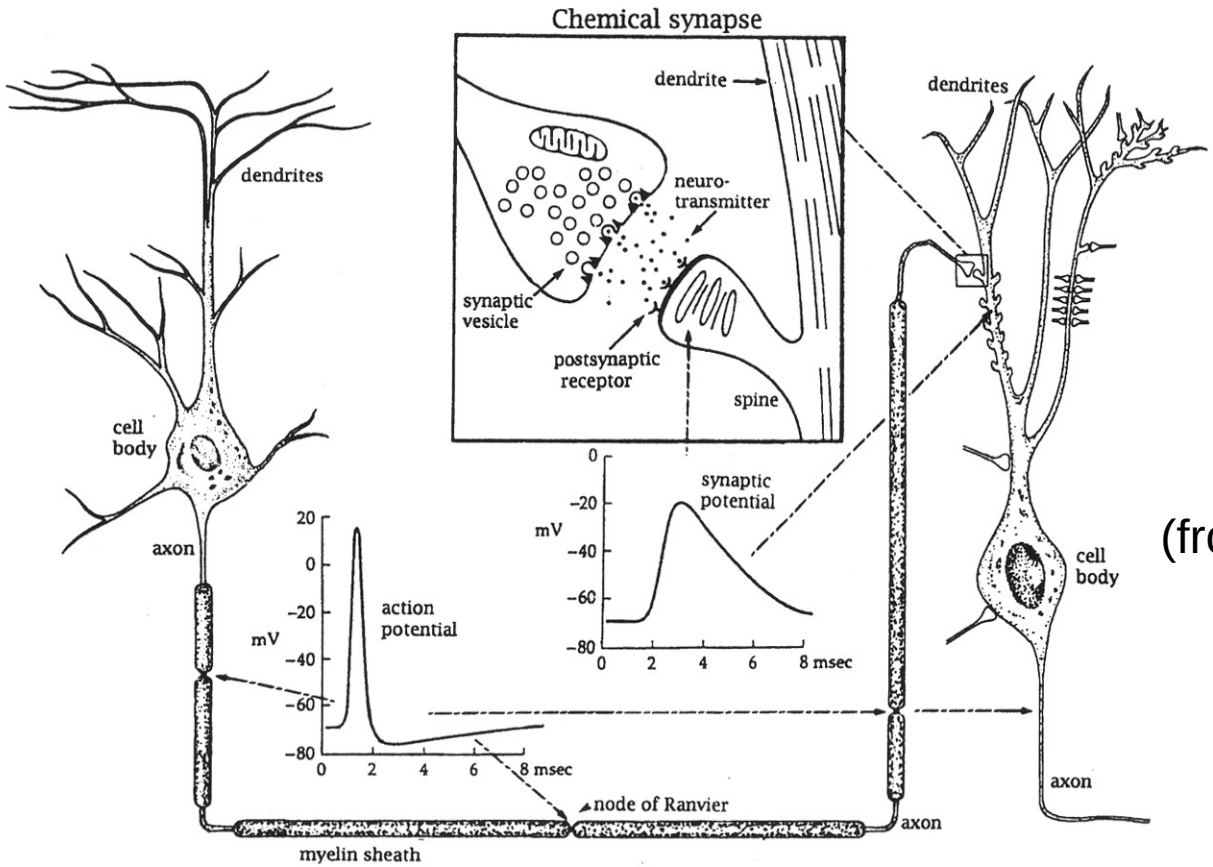
- Ionic movement can be described in terms of either particle flow or electrical current.
- The conversion factor is Faraday's constant:

$$F = \left(6.02 \times 10^{23}\right) \frac{\text{particles}}{\text{mole}} \times \left(1.6 \times 10^{-19}\right) \frac{\text{columbs}}{\text{particle}} = 96,487 \ \frac{\text{columbs}}{\text{mole}}$$

Excitable cells:

- Cells that can generate electrical potentials and currents are referred to as *excitable cells*.
- These potentials and currents can be observed in the cells' interior volume, across their membranes, and in their surrounding conducting volume.
- Excitable cells include *nerve cells* (*neurons*), *muscle fibers*, and *sensory receptor* (*transducer*) *cells*.

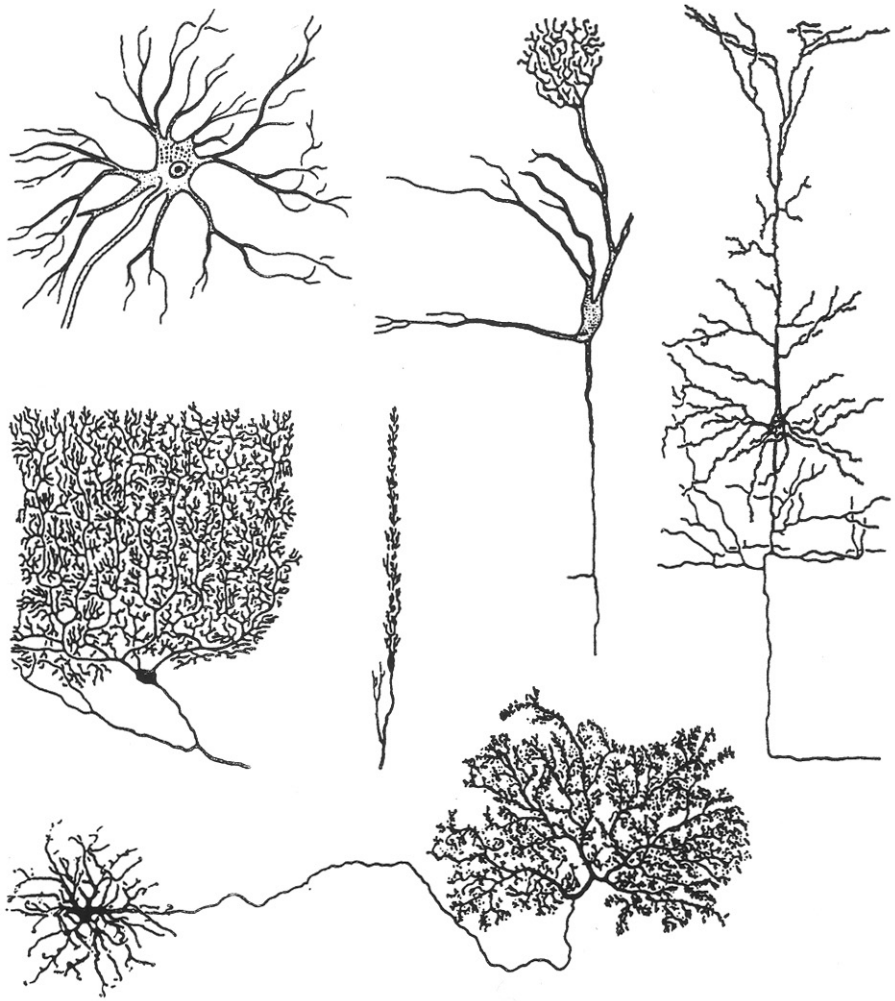
Nerve cells:



(from Johnston and Wu)

Figure 1.2 Neurons convey information by electrical and chemical signals. Electrical signals travel from the cell body of a neuron (left) to its axon terminal in the form of action potentials. Action potentials trigger the secretion of neurotransmitters from synaptic terminals (upper insert). Neurotransmitters bind to postsynaptic receptors and cause electric signals (synaptic potential) in the postsynaptic neuron (right). Synaptic potentials trigger action potentials, which propagate to the axon terminal and trigger secretion of neurotransmitters to the next neuron. (Adapted from Kandel et al. 1991 and from L.L. Iversen, copyright © 1979 by Scientific American, Inc. All rights reserved.)

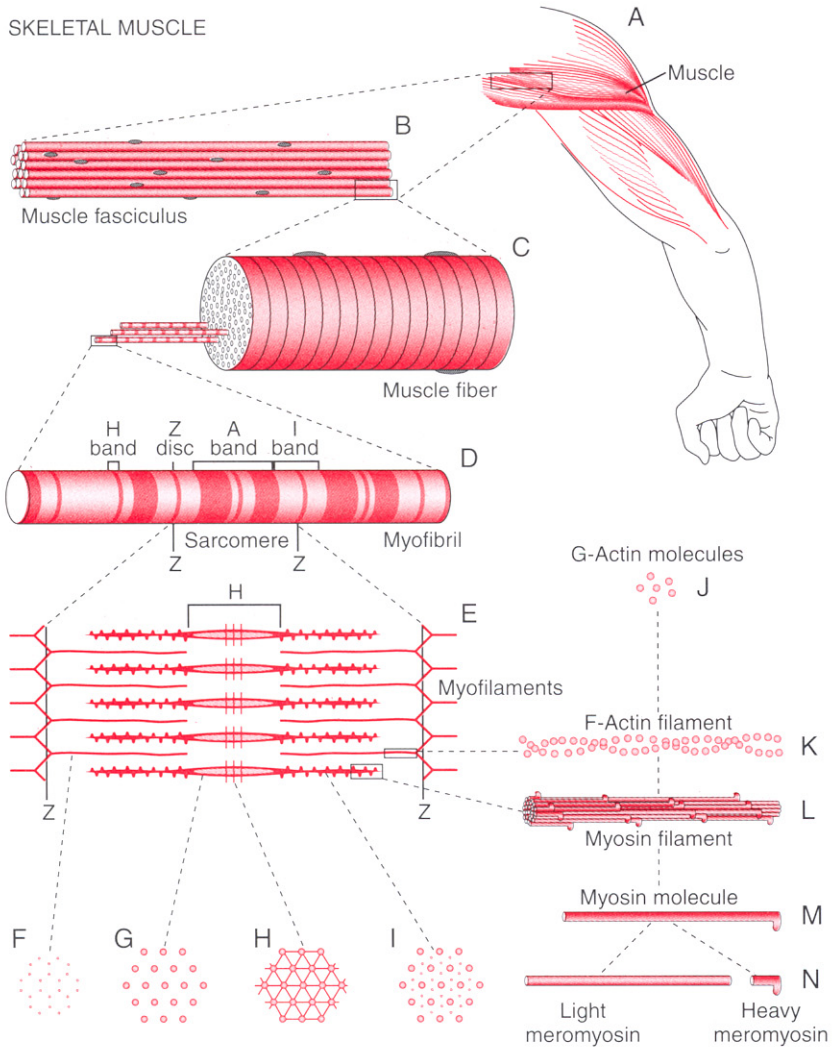
Examples of neuron morphologies:



(from Johnston and Wu)

Figure 1.1 Examples of neurons in the nervous system exhibiting various morphology. From the upper left in clockwise order: motor neuron from the spinal cord, mitral cell from olfactory bulb, pyramidal cell from cortex, horizontal cell from retina, and Purkinje cell (front and side views) from cerebellum. (From Nicholls et al. 1992 and Fisher and Boycott 1974.)

Skeletal muscle cells:

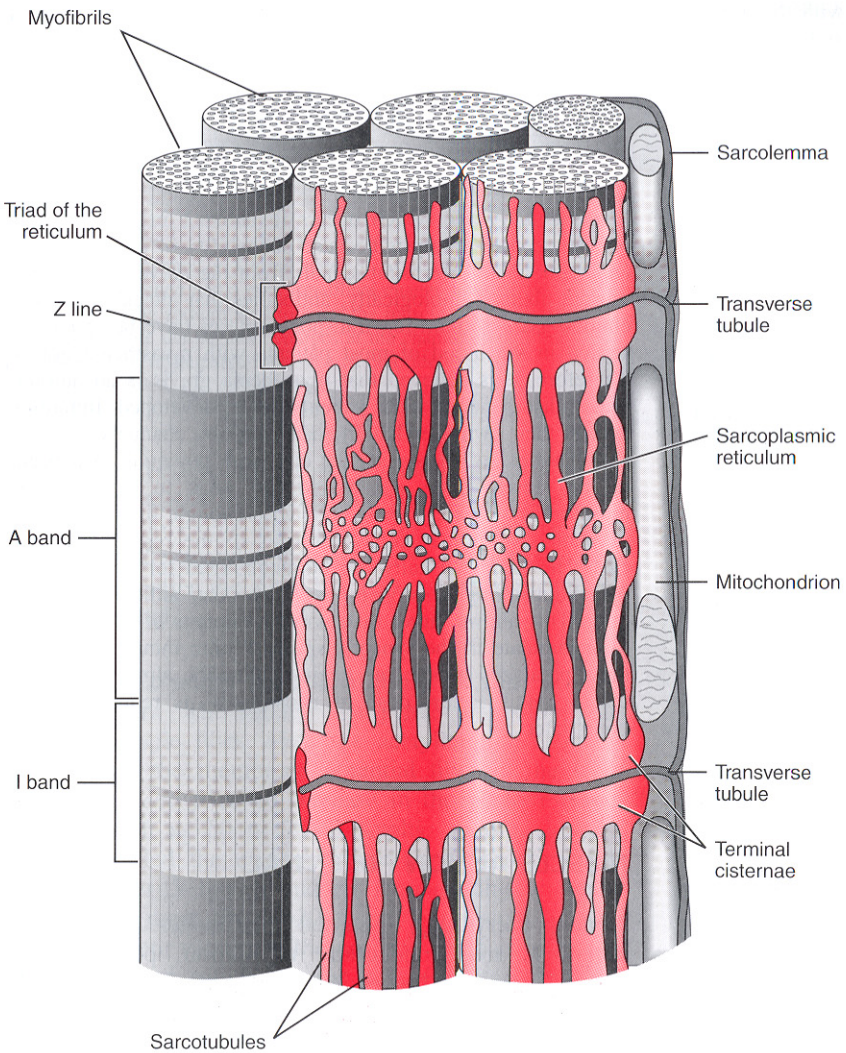


(from Guyten)

FIGURE 6–1

Organization of skeletal muscle, from the gross to the molecular level. *F*, *G*, *H*, and *I* are cross sections at the levels indicated. (Drawing by Sylvia Colard Keene. Modified from Fawcett DW: Bloom and Fawcett: A Textbook of Histology. Philadelphia: WB Saunders Co, 1986.)

Transverse tubule-sarcoplasmic reticulum system for excitation-contraction coupling:



(from Guyten)

FIGURE 7–5

Transverse (T) tubule-sarcoplasmic reticulum system. Note the *longitudinal tubules* that terminate in large *cisternae*. The cisternae in turn abut the T tubules. Note also that the T tubules communicate with the outside of the cell membrane. This illustration was drawn from frog muscle, which has one T tubule per sarcomere, located at the Z line. A similar arrangement is found in mammalian heart muscle, but mammalian skeletal muscle has two T tubules per sarcomere, located at the A-I junctions. (Redrawn from Bloom W, Fawcett DW: A Textbook of Histology. Philadelphia: WB Saunders Co, 1986. Modified after Peachey LD: J Cell Biol 25:209, 1965. Drawn by Sylvia Colard Keene.)

Cardiac muscle cells are interconnected via electrical "gap junctions":

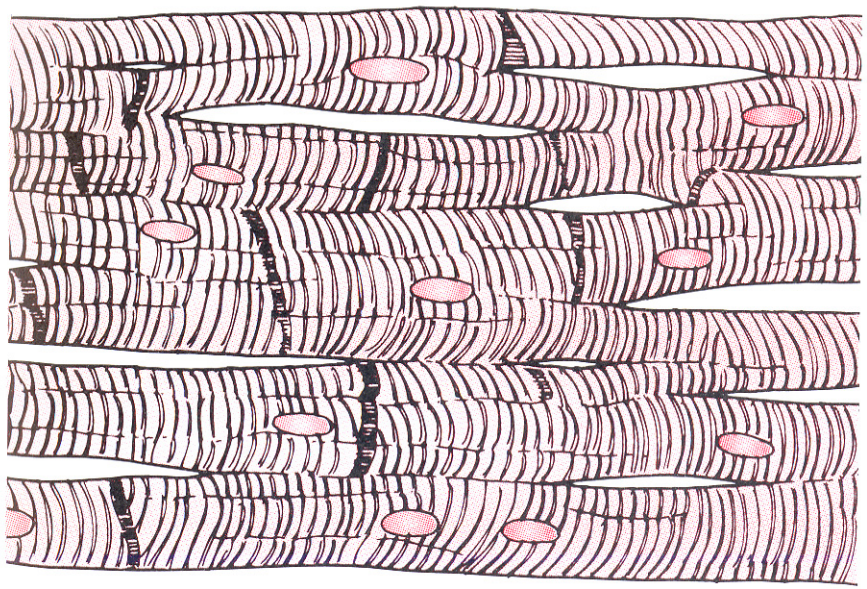


FIGURE 9 – 2

"Syncytial," interconnecting nature of cardiac muscle fibers.

(from Guyten)

Electrical "gap junctions" in cardiac cells:

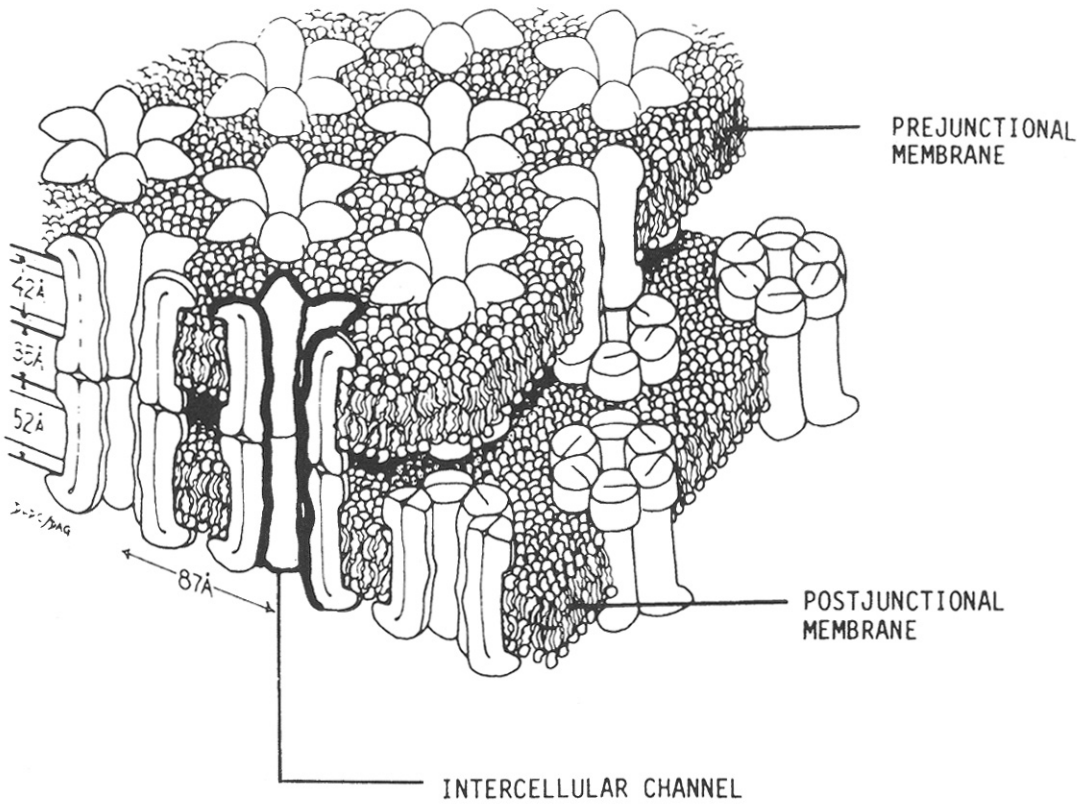


Figure 9.4. Details of the communicating-type intercellular cardiac junction (connexon array) is shown. Each unit (connexon) is a protein channel running transverse to the opposing membranes. Connexons from abutting cells align themselves to form structural continuity. The structural detail shown is based on morphometry obtained from X-ray diffraction, electron microscopy, and chemical studies. The gap spacing is given as 35 Å. [R. Plonsey, The use of a bidomain model for the study of excitable media, *Lectures on Mathematics in the Life Sciences* **21**:123–149 (1989). From L. Makowski, D. L. D. Caspar, W.C. Phillips, and D. A. Goodenough, Gap junctional structures II. Analysis of x-ray diffraction, *J. Cell Biol.* **74**:629–645 (1977). Reproduced from the Journal of Cell Biology, 1977, vol. 74, pp. 629–645 by copyright permission of the Rockefeller University Press.]

Sensory receptors:

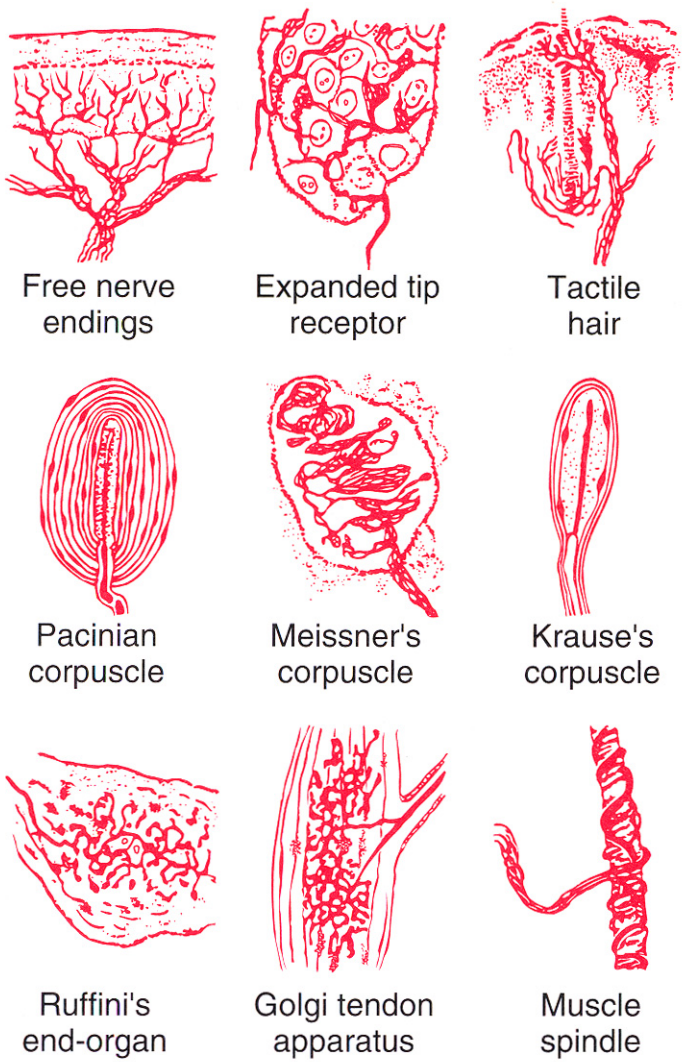


FIGURE 46–1

Several types of somatic sensory nerve endings.

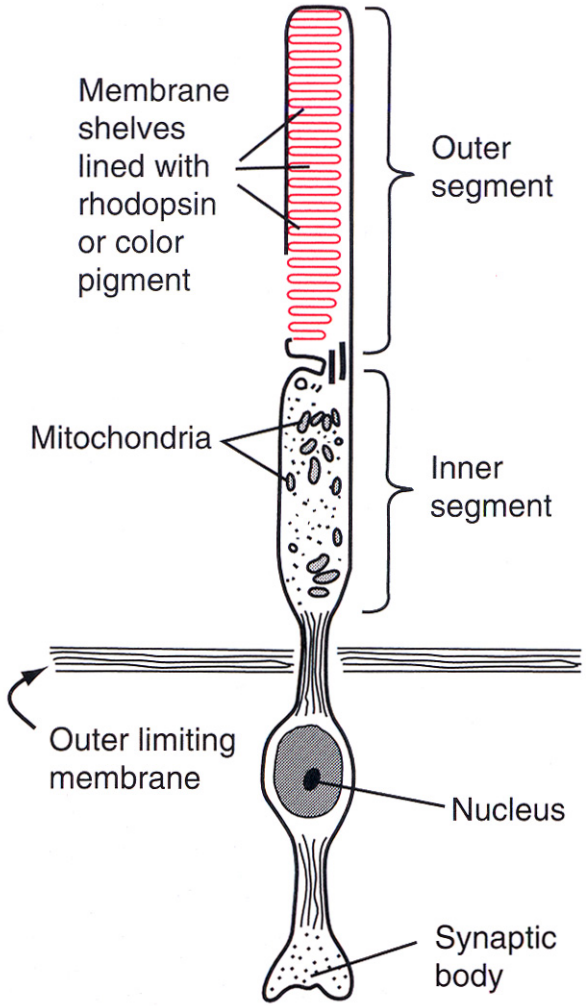


FIGURE 50–3

Schematic drawing of the functional parts of the rods and cones.

(from Guyten)

Sensory receptors (cont.):

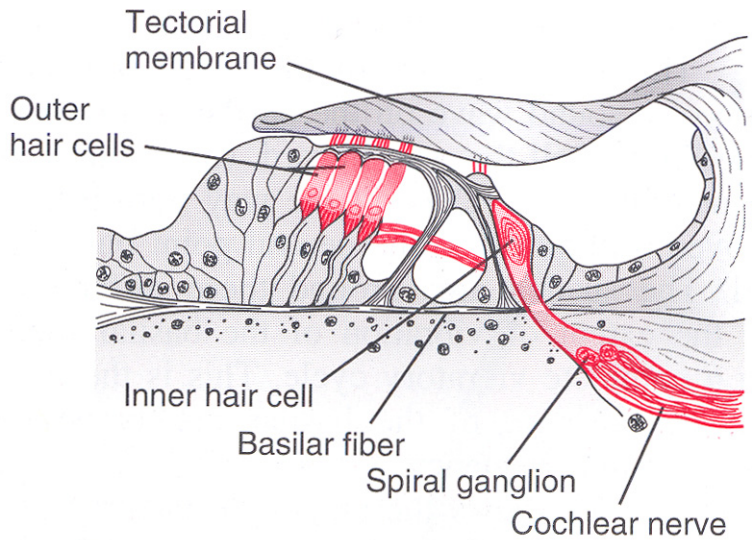


FIGURE 52–7

Organ of Corti, showing especially the hair cells and the tectorial membrane against the projecting hairs.

(from Guyten)

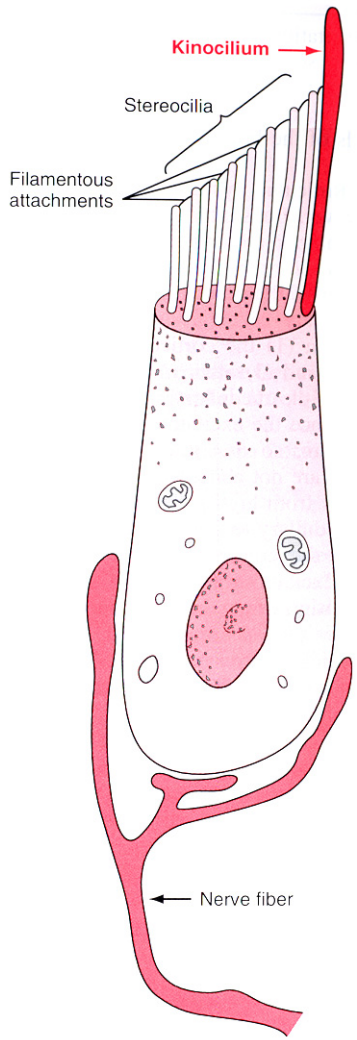


FIGURE 55–10

Hair cell of the equilibrium apparatus and its synapses with the vestibular nerve.

Sensory receptors (cont.):

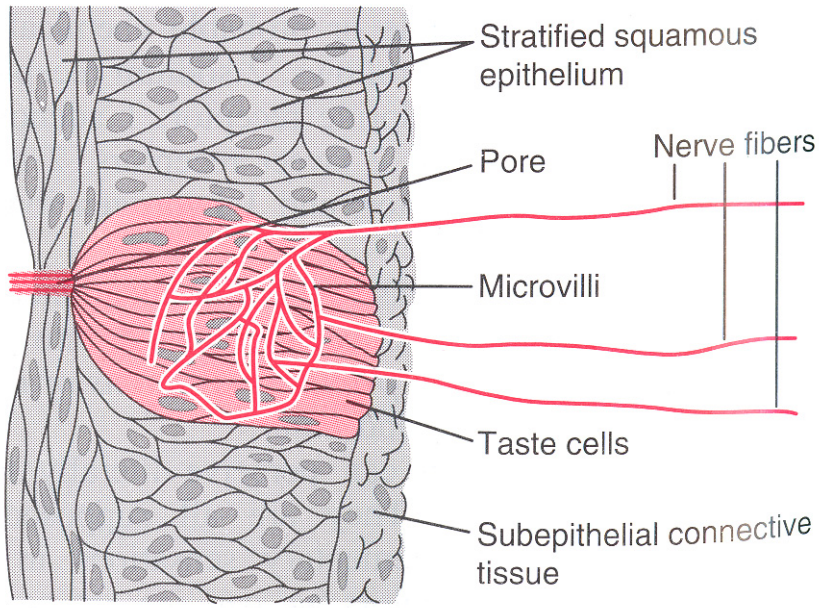


FIGURE 53–1

Taste bud.

(from Guyten)

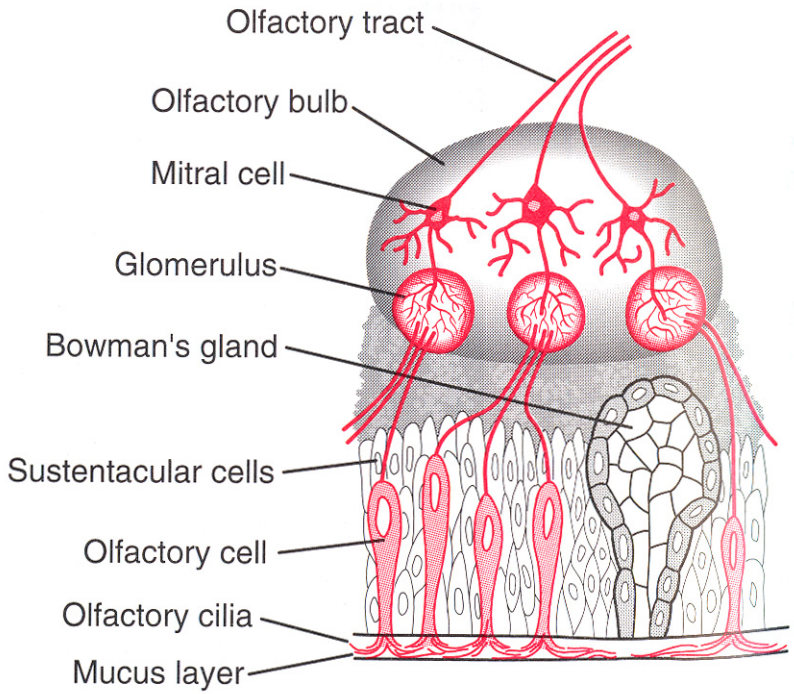


FIGURE 53–3

Organization of the olfactory membrane and olfactory bulb of the superlying brain.

2. EQUIVALENT ELECTRICAL CIRCUITS FOR CELL MEMBRANES

We will look at:

- Ionic composition of excitable cells
- Nernst-Planck equation
- Membrane structure
- Nernst potential
- Parallel-conductance model

Ionic composition of excitable cells:

- *Sodium* (Na^{+}) and *potassium* (K^{+}) are the most important ions for the electrical activity of the majority of excitable cells.
- *Calcium* (Ca^{2+}) and *chloride* (Cl^{-}) play a significant role in some circumstances.
- Many of the fundamental properties of ionic movement are the same no matter which ion is being considered.

  Consequently, we will often derive mathematical expressions for "the p^{th} ion".

Ionic composition (cont.):

- Example intra- and extra-cellular ionic concentrations are given below.

Table 3.1. Ionic Concentrations[a]

	Concentration (nM/l)			
	Muscle (frog)		Nerve (squid axon)	
	Intracellular	Extracellular	Intracellular	Extracellular
K^+	124	2.2	397	30
Na^+	4	109	50	437
Cl^-	1.5	77	40	556
A^-	126.5			

[a]The A^- ion is large and impermeable.

- Note that the particular ratios of intra- to extra-cellular ionic concentrations are similar across different types of excitable cells.

Nernst-Planck Equation:

- The *Nernst-Planck* equation describes the effects of spatial differences in *concentration* and/or *electric potential* on ion flow.
- The individual effect of a concentration gradient is described by *Fick's law of diffusion*.
- The individual effect of an electric potential gradient is described by *Ohm's law of drift*.

Fick's law of diffusion:

$$\bar{j}_d = -D\nabla C, \qquad (3.1)$$

where:

$\bar{j}_d$ is the flux due to diffusion
D is the diffusion coefficient
C is the concentration as a function of position
∇ is the *Del operator*:

$$\nabla \equiv \bar{a}_x \frac{\partial}{\partial x} + \bar{a}_y \frac{\partial}{\partial y} + \bar{a}_z \frac{\partial}{\partial z} \qquad (1.17)$$

The diffusion coefficient:

The diffusion coefficient is also known as *Fick's coefficient*, or alternatively as the diffusion or Fick's *constant*. However, it is not strictly a constant because it varies with temperature and somewhat with C.

D is normally determined empirically.

Table 3.2. Numerical Values for Several Diffusion Coefficients

Ion	D	Units	Conditions	Reference
Na^+	1.33×10^{-5}	cm^2/sec	at 25 °C	3
K^+	1.96×10^{-5}	cm^2/sec	at 25 °C	3
Cl^-	2.03×10^{-5}	cm^2/sec	at 25 °C	3
KCl	2.03×10^{-5}	cm^2/sec	0.002 mole/l, 25 °C	4
NaCl	1.58×10^{-5}	cm^2/sec	0.002 mole/l, 25 °C	4

Ohm's law of drift:

$$\bar{j}_e = -u_p \frac{Z_p}{|Z_p|} C_p \nabla \Phi, \qquad (3.2)$$

where:

$\bar{j}_e$	is the ionic flux due to an electric field
$-\nabla\Phi$	is the electric field
u_p	is the mobility of the p$^{\text{th}}$ ion
$Z_p/\left\|Z_p\right\|$	is the sign of the valence of the p$^{\text{th}}$ ion
C_p	is the concentration of the p$^{\text{th}}$ ion

Relating diffusion and drift:

Diffusion and drift are impeded by the same molecular processes, i.e., collisions with solvent molecules, and consequently a physical connection exists between the parameters u_p and D.

The mathematical expression for this relationship is known as *Einstein's equation*.

Einstein's equation:

$$D_p = \frac{u_p RT}{|Z_p| F}, \qquad (3.3)$$

where:

R is the gas constant
T is the absolute temperature
F is Faraday's constant

Table 3.3. Numerical Values for Faraday's Constant *F* and the Gas Constant R

Constant	Value
F	96,487 coulombs/mole
R	8.314 joules/K mole
RT/F	8.314 × .300/96487 = 25.8 mV at 27 °C

Total ion flow:

The total flux when both diffusional and electric field forces are present is:

$$\bar{j}_p = \bar{j}_d + \bar{j}_e \tag{3.4}$$

$$= -D_p \left(\nabla C_p + \frac{Z_p C_p F}{RT} \nabla \Phi \right), \tag{3.5}$$

which is known as the **Nernst-Planck equation**.

Electric current density:

The electric current density can be found by multiplying the ionic flux by FZ_p, giving:

$$\bar{J}_p = -D_p F Z_p \left(\nabla C_p + \frac{Z_p C_p F}{RT} \nabla \Phi \right), \quad (3.6)$$

Alternatively, substituting for D_p using Einstein's equation, one has:

$$\bar{J}_p = -u_p \left(RT \frac{Z_p}{|Z_p|} \nabla C_p + |Z_p| C_p F \nabla \Phi \right). \quad (3.7)$$

Resistance and conductance:

The linear relationship between the current density and the strength of the electric field applied to an electrolyte suggests that an expression can be derived for the *resistance* (or its reciprocal, *conductance*) of the intra- or extra-cellular space.

Considering a standard form for Ohm's law:

$$\bar{J} = \sigma \bar{E},$$

how does the electrical conductivity σ relate to parameters such as mobility and concentration?

Conductivity:

The electric current density arising solely under the influence of an electric field is:

$$\bar{J}_p^e = -u_p \left|Z_p\right| C_p F \nabla \Phi. \tag{3.8}$$

For example, a KCl electrolyte in which there is complete dissociation has the total current density:

$$\bar{J}_{\mathsf{KCl}}^e = F C_{\mathsf{KCl}} \left[u_{\mathsf{K}} + u_{\mathsf{Cl}}\right] \bar{E}, \tag{3.9}$$

giving the electrolyte conductivity:

$$\sigma = F C_{\mathsf{KCl}} \left[u_{\mathsf{K}} + u_{\mathsf{Cl}}\right]. \tag{3.10}$$

Membrane structure:

- Excitable cells are surrounded by a *plasma membrane* consisting of a lipid bilayer.
- The passage of ions through the membrane is regulated by:

1. Pumps and exchangers

2. Channels

Membrane structure (cont.):

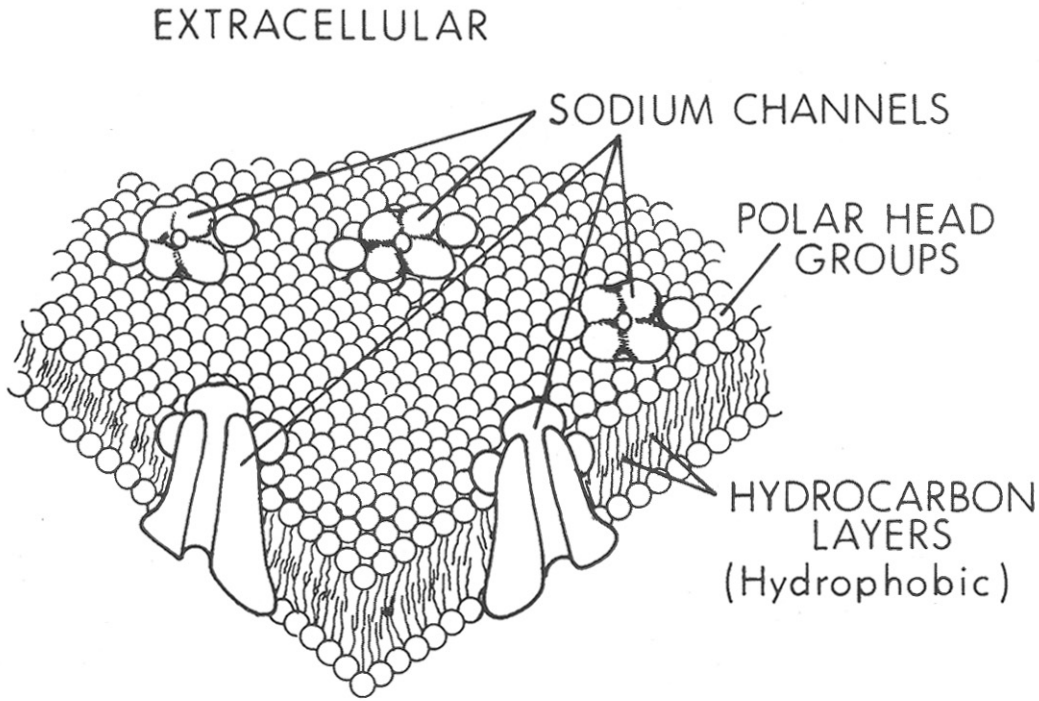


Figure 3.1. Schematic representation of the model of membrane structure showing sodium channel proteins embedded in the lipid bilayer matrix of the membrane. The channel density is unphysiologically high, for illustrative purposes. [Drawing based on W. A. Catterall et al., "Structure and modulation of voltage-gated sodium channels" in "Ion Channels in the Cardiovascular System," P. M. Spooner and A. M. Brown, eds., Futura, Armonk, New York, 1994.

Pumps and exchangers:

- Pumps are active processes (i.e., they consume energy) that move ions against the concentration gradients.
- Exchangers use the concentration gradient of one ion to move another ion against its concentration gradient.
- The purpose of pumps and exchangers is to maintain the different intra- and extra-cellular ionic concentrations.
- The major ion transporters are: $\mathrm{Na^+}$-$\mathrm{K^+}$ pump, $\mathrm{Na^+}$-$\mathrm{Ca^{2+}}$ exchanger, $\mathrm{Ca^{2+}}$ pump, $\mathrm{Bicarbonate}$-$\mathrm{Cl^-}$ exchanger, $\mathrm{Cl^-}$-$\mathrm{Na^+}$-$\mathrm{K^+}$ cotransporter.

Channels:

- Channels are passive processes that allow ions to pass through the membrane under the influence of concentration and electric potential gradients.
- Channels exhibit *selective permeability*, i.e., they only allow certain ions to pass through them.
- Ion channel *gates* regulate the permeability of channels, allowing control over the flow of particular ions.

Membrane capacitance:

- The lipid membrane itself has a specific resistance of $10^9\ \Omega{\cdot}\mathrm{cm}^2$, i.e., it is effectively an *insulator*.
- Consequently, charge can build up on each side of the membrane in regions where there are no channels or where channels are closed.
  Because of the thinness of the membrane, it acts as a *capacitor*, with a capacitance typically around $C_m = 0.9\ \mu\mathrm{F}/\mathrm{cm}^2$.

Ion flow through open channels:

- From the Nernst-Planck equation, the flow of the p^{th} ion will depend on both the concentration gradient of the p^{th} ion and an electric potential gradient.
- For an excitable cell, the unequal concentration of ions in the intra- versus extra-cellular spaces produces ion flow through any open ion channels.
- Ions will accumulate on the membrane because of its capacitance, producing an electrical field across and within the membrane that will in turn exert a force on all charged particles within ion channels.

Nernst equilibrium:

A *Nernst equilibrium* is achieved for a particular ion when the electric field force exactly counteracts the force of the concentration gradient for that ion, such that the net flow through an ion channel is zero:

$$\bar{J}_p = 0 = -D_p F Z_p \left[\nabla C_p + \frac{Z_p C_p F}{RT} \nabla \Phi \right], \quad (3.15)$$

and hence:

$$\nabla C_p = -\frac{Z_p C_p F}{RT} \nabla \Phi. \quad (3.16)$$

Nernst equilibrium (cont.):

Assuming that the concentration and electric potential gradients only act in the direction x, perpendicular to the membrane surface, this simplifies to:

$$\frac{\mathrm{d}C_p}{\mathrm{d}x} = -\frac{Z_p C_p F}{RT}\frac{\mathrm{d}\Phi}{\mathrm{d}x} \tag{3.17}$$

$$\Rightarrow \frac{\mathrm{d}C_p}{C_p} = -\frac{Z_p F}{RT}\mathrm{d}\Phi. \tag{3.18}$$

Nernst equilibrium (cont.):

Integrating across the membrane from the extracellular space e to the intracellular space i:

$$\int_e^i \frac{\mathrm{d}C_p}{C_p} = -\frac{Z_p F}{RT} \int_e^i \mathrm{d}\Phi \qquad (3.19)$$

gives:

$$\ln\left(\frac{[C_p]_i}{[C_p]_e}\right) = -\frac{Z_p F}{RT} \left\{\Phi_i - \Phi_e\right\}, \qquad (3.20)$$

where $\ln \equiv \log_e$.

Nernst potential:

Thus the potential difference across the membrane at equilibrium, referred to as the **Nernst potential**, is:

$$V_m^{eq} = \Phi_i - \Phi_e = \frac{-RT}{Z_p F} \ln\left(\frac{[C_p]_i}{[C_p]_e}\right), \quad (3.21)$$

where the transmembrane potential V_m is defined as the intracellular potential Φ_i minus the extracellular potential Φ_e.

Nernst potential (cont.):

In the case where the temperature is 20°C, the Nernst potential is:

$$V_m^{eq} = E_p = \frac{-25}{Z_p} \ln\left(\frac{[C_p]_i}{[C_p]_e}\right) \text{ mV}$$

$$= \frac{25}{Z_p} \ln\left(\frac{[C_p]_e}{[C_p]_i}\right) \text{ mV}, \qquad (3.22)$$

or using base 10 instead of the natural logarithm:

$$V_m^{eq} = E_p = \frac{58}{Z_p} \log_{10}\left(\frac{[C_p]_e}{[C_p]_i}\right) \text{ mV}. \quad (3.23)$$

Equilibrium/reversal potentials:

The Nernst potential for a particular ion is often referred to as the *equilibrium potential* and is given the symbol E_p.

For example, the equilibrium potentials for sodium and potassium ions are given the symbols E_{Na} and E_{K}, respectively.

The equilibrium potential is also sometimes referred to as the *reversal potential*, because at this potential the direction of the ionic current reverses from inwards to outwards, or vice versa.

Example equilibrium/reversal potentials:

Table 2.1 Ion concentrations and equilibrium potentials

	Inside (mM)	Outside (mM)	Equilibrium Potential (NE) $E_i = \frac{RT}{zF} \ln \frac{[C]_{out}}{[C]_{in}}$
Frog muscle (Conway 1957)			$T = 20°C = 293°K$
K^+	124	2.25	$58 \log \frac{2.25}{124} = -101$ mV
Na^+	10.4	109	$58 \log \frac{109}{10.4} = +59$ mV
Cl^-	1.5	77.5	$-58 \log \frac{77.5}{1.5} = -99$ mV
Ca^{2+}	$4.9^\dagger$	2.1	$29 \log \frac{2.1}{10^{-4}} = +125$ mV
Squid axon (Hodgkin 1964)			
K^+	400	20	$58 \log \frac{20}{400} = -75$ mV
Na^+	50	440	$58 \log \frac{440}{50} = +55$ mV
Cl^-	40–150	560	$-58 \log \frac{560}{40-150} = -66 - (-33)$ mV
Ca^{2+}	$0.4^\dagger$	10	$29 \log \frac{10}{10^{-4}} = +145$ mV
Typical mammalian cell			$T = 37°C = 310°K$
K^+	140	5	$62 \log \frac{5}{140} = -89.7$ mV
Na^+	5–15	145	$62 \log \frac{145}{5-15} = +90.7 - (+61.1)$ mV
Cl^-	4	110	$-62 \log \frac{110}{4} = -89$ mV
Ca^{2+}	$1\text{–}2^\dagger$	2.5–5	$31 \log \frac{2.5-5}{10^{-4}} = +136 - (+145)$ mV

$^\dagger(10^{-4})$ free

(from Johnston and Wu)

Parallel conductance model:

- The two major results developed thus far are the *Nernst-Planck equation* and the *Nernst potential*.
- The latter utilizes the former to derive an expression for the equilibrium potential of each ion species.
- We would like to apply the Nernst-Planck equation to ion flow through a channel when it is *not* at the Nernst equilibrium. However, the exact concentration and electric potential gradients along the length of a channel are typically not known.

Goldman-Hodgkin-Katz (GHK) model:

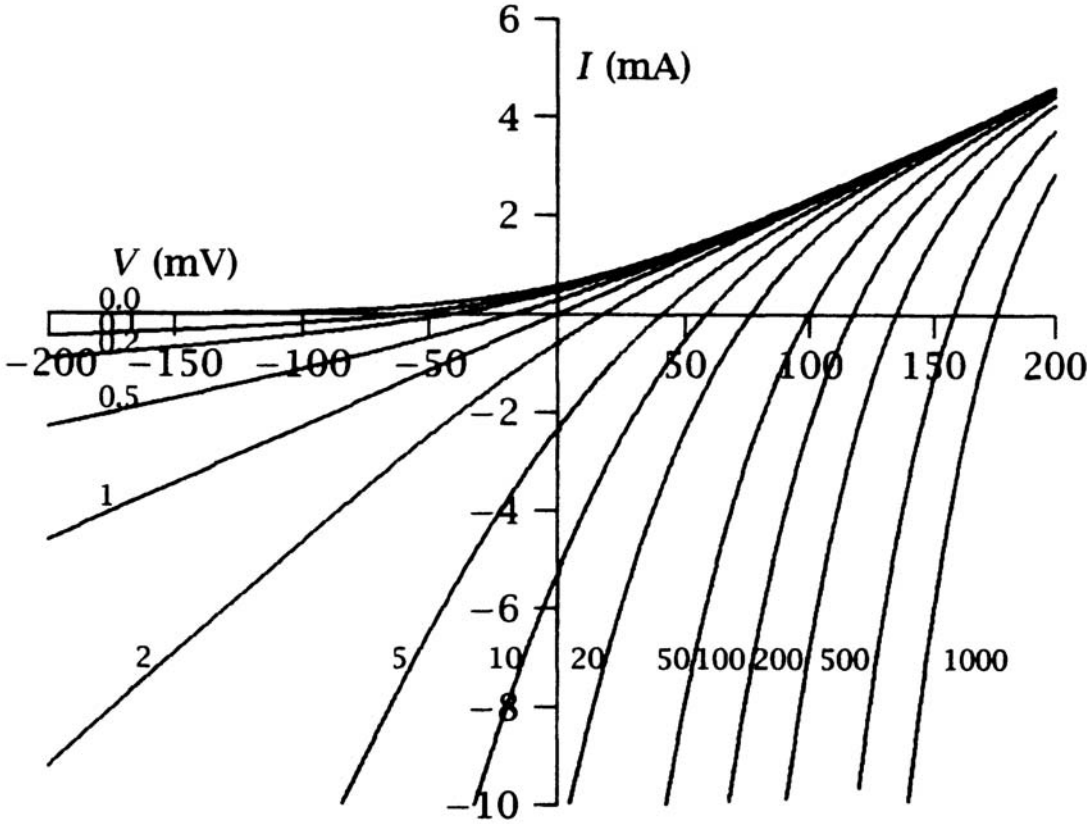


Figure 2.5 Current-voltage relations given by equation 2.7.17 (GHK current equation) for various values of $[C]_{out}/[C]_{in}$ (indicated by small numbers near each curve).

(from Johnston and Wu)

Parallel conductance model (cont.):

- Unfortunately the GHK equations do *not* accurately describe the behaviour of most ion channels.
- Consequently, a phenomenological description of current flow in ionic channels is typically used. This **parallel conductance model** does incorporate three earlier results:
  1. the *capacitance* of the plasma membrane,
  2. the *conductive* nature of ion flow, and
  3. the *equilibrium potential* for each ion.

Parallel conductance model (cont.):

Assuming independent conductance channels for K^+, Na^+ and Cl^-, the electric circuit for a *membrane patch* is:

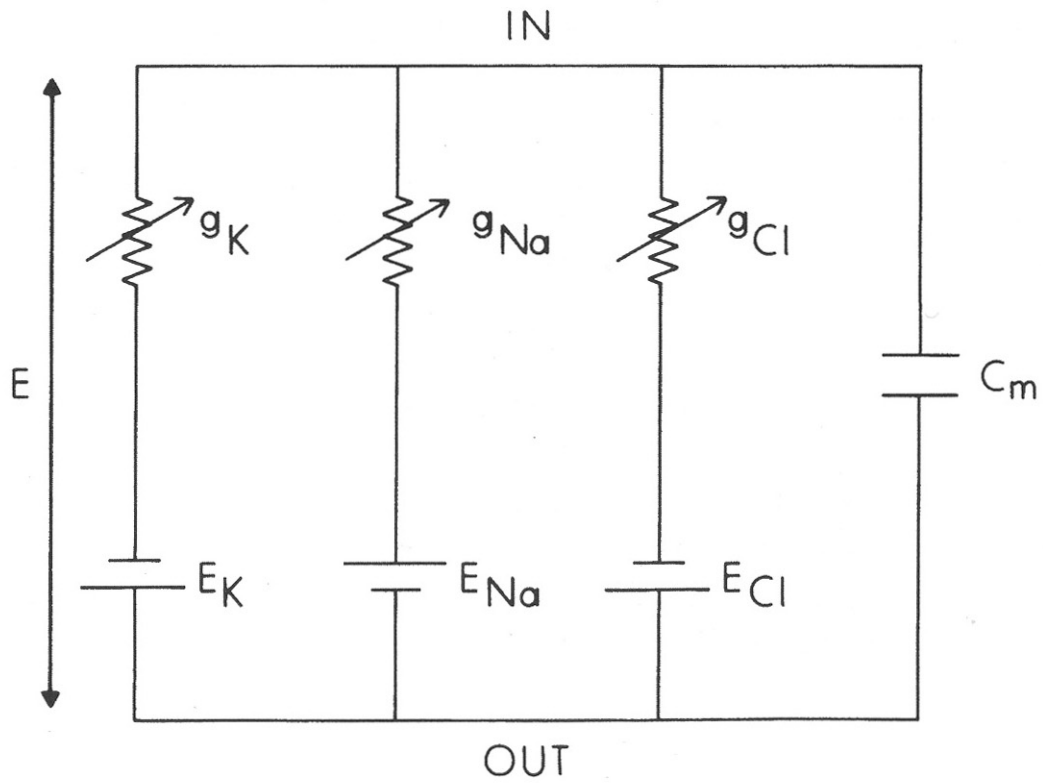


Figure 3.3. Parallel-conductance model of an excitable membrane, for which one assumes independent conductance channels for K^+, Na^+, and Cl^-. The battery polarity is chosen to show that the nominal Nernst potential of E_K and E_{Cl} is negative and E_{Na} positive.

Ionic currents:

The current for the p^{th} ion is assumed to be proportional to how far the membrane potential V_m deviates from the equilibrium potential E_p, with the constant of proportionality g_p corresponding to the instantaneous conductance of the channel.

For the three ionic channels shown in Fig. 3.3, we have:

$$I_{\mathsf{K}} = g_{\mathsf{K}} (V_m - E_{\mathsf{K}}) \tag{3.26}$$

$$I_{\mathsf{Na}} = g_{\mathsf{Na}} (V_m - E_{\mathsf{Na}}) \tag{3.27}$$

$$I_{\mathsf{Cl}} = g_{\mathsf{Cl}} (V_m - E_{\mathsf{Cl}}) \tag{3.28}$$

Capacitive current:

The capacitive current is:

$$I_C = C\frac{\mathrm{d}V_m}{\mathrm{d}t}, \tag{3.29}$$

where C is the capacitance for the patch of membrane.

Importantly, at rest (i.e., at steady state), $I_C = 0$ because $\mathrm{d}V_m/\mathrm{d}t = 0$.

Resting V_m at steady-state:

The total transmembrane current is:

$$I_m = I_C + I_{\mathsf{K}} + I_{\mathsf{Na}} + I_{\mathsf{Cl}}.$$

Assuming that no current is being injected into the intra- or extra-cellular space, the total transmembrane current must be zero, such that at steady state:

$$I_m = 0 = 0 + I_{\mathsf{K}} + I_{\mathsf{Na}} + I_{\mathsf{Cl}}$$

$$\Rightarrow g_{\mathsf{K}}\left(V_m - E_{\mathsf{K}}\right) + g_{\mathsf{Na}}\left(V_m - E_{\mathsf{Na}}\right) + g_{\mathsf{Cl}}\left(V_m - E_{\mathsf{Cl}}\right) = 0. \qquad (3.30)$$

Resting V_m at steady-state (cont.):

Solving for V_m to obtain the *resting transmembrane potential* V_{rest} gives:

$$V_{\text{rest}} = \frac{g_{\text{K}} E_{\text{K}} + g_{\text{Na}} E_{\text{Na}} + g_{\text{Cl}} E_{\text{Cl}}}{g_{\text{K}} + g_{\text{Na}} + g_{\text{Cl}}}. \quad (3.31)$$

That is, the resting membrane potential is the weighted sum of the equilibrium potentials, where the weightings depend on the resting values of the ionic conductances.

Example resting V_m:

Assuming the following equilibrium potentials and resting ionic conductances for the squid axon:

$$\begin{aligned} E_{\mathrm{K}} &= -74.7\ \mathrm{mV}, & g_{\mathrm{K}} &= 0.367\ \mathrm{mS/cm^2}, \\ E_{\mathrm{Na}} &= \ \ 54.2\ \mathrm{mV}, & g_{\mathrm{Na}} &= 0.010\ \mathrm{mS/cm^2}, \\ E_{\mathrm{Cl}} &= -65.8\ \mathrm{mV}, & g_{\mathrm{Cl}} &= 0.582\ \mathrm{mS/cm^2}, \end{aligned}$$

from Eqn. (3.31) we find that the resting membrane potential is $V_m = -68.0\ \mathrm{mV}$.

Membrane conductance/resistance at rest:

If the membrane potential is at rest, then the total resting membrane conductance G (or its reciprocal, the total resting membrane resistance R) can be determined from the resting values of the ionic conductances according to:

$$G = \frac{1}{R} = g_{\mathrm{K}} + g_{\mathrm{Na}} + g_{\mathrm{Cl}}.$$

Equivalent circuit near rest:

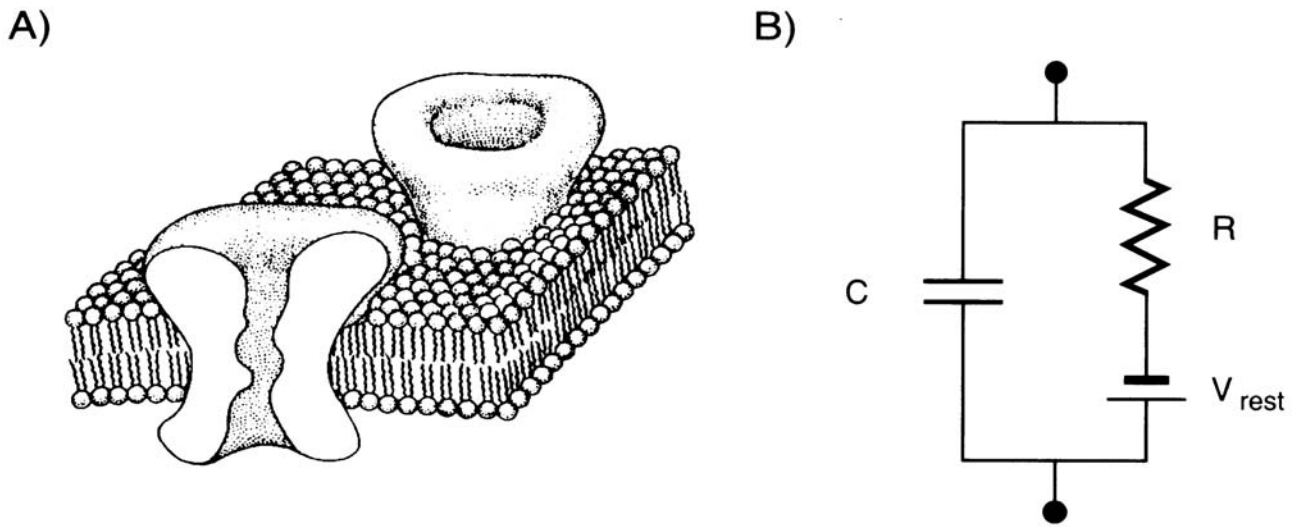


Fig. 1.1 NATURE OF THE PASSIVE NEURONAL MEMBRANE **(A)** Schematic representation of a small patch of membrane of the types enclosing all cells. The 30–50 Å thin bilayer of lipids isolates the extracellular side from the intracellular one. From an electrical point of view, the resultant separation of charge across the membrane acts akin to a capacitance. Proteins inserted into the membrane, here ionic channels, provide a conduit through the membrane. Reprinted by permission from Hille (1992). **(B)** Associated lumped electrical circuit for this patch, consisting of a capacitance and a resistance in series with a battery. The resistance mimics the behavior of voltage-independent ionic channels inserted throughout the membrane and the battery accounts for the cell's resting potential V_{rest}.

(from Koch)

Channel structure:

The coarse structure of *membrane channel proteins* can be determined by electron microscopy or X-ray diffraction.

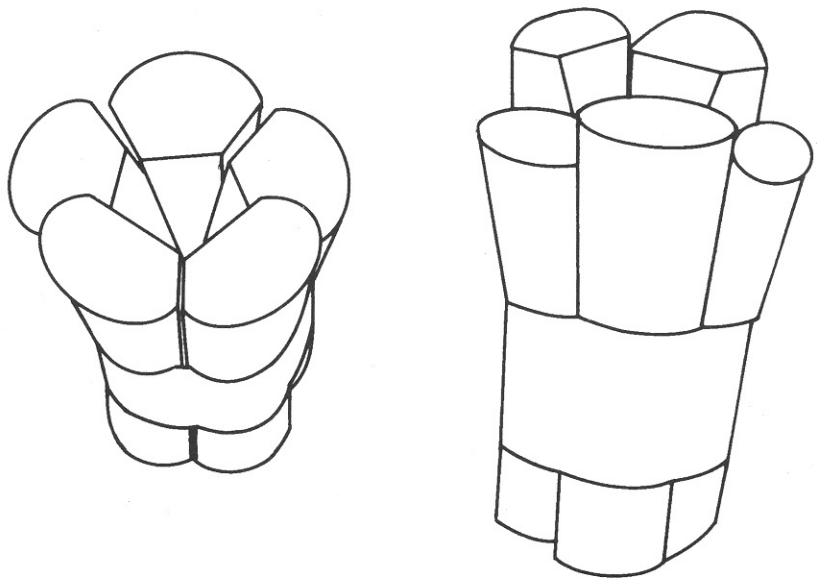


Figure 4.1. A model of the acetylcholine receptor which shows the five component subunits and the aqueous pore. The band locates the membrane bilayers through which the molecule passes; the lower part is cytoplasmic. [From R. M. Stroud and J. Finer-Moore, Acetylcholine receptor structure, function, and evolution. Reproduced with permission from *Annu. Rev. Cell Biol.* **1**:317–351 (1985). Copyright 1985, Annual Reviews, Inc.]

Channel structure (cont.):

- *Molecular genetics* can be used to express a channel protein in a cell that does not normally make that protein.
- The resulting channel properties can be evaluated to determine whether the protein synthesized is indeed the desired protein.

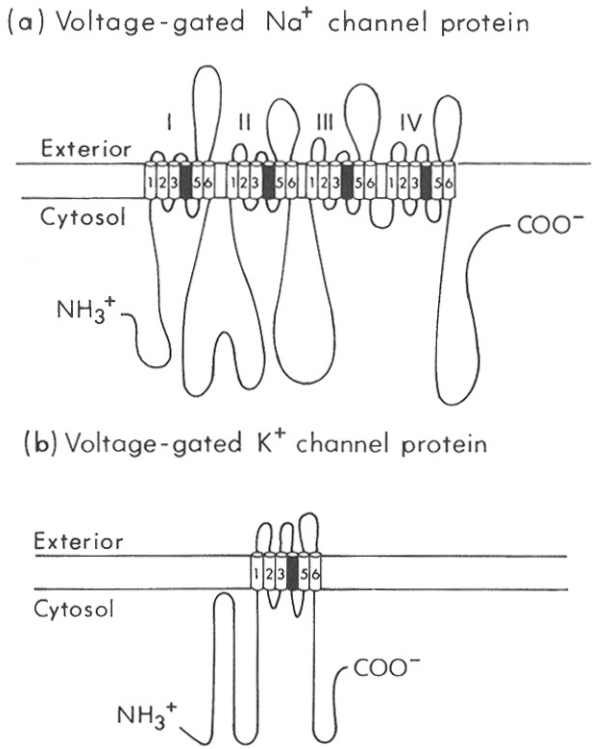


Figure 4.2. Proposed transmembrane structure of (a) voltage-gated Na^+ channel protein and (b) voltage-gated K^+ channel protein. The sodium channel arises from a single gene; it contains 1800–2000 amino acids, depending on the source. About 29 percent of the residues are identical to those in the voltage-gated Ca^{++} channel protein. There are four homologous domains indicated by the Roman numerals. Each of these is thought to contain six transmembrane α helices (Arabic numerals). The helix number 4 in each domain is thought to function as a voltage sensor. The shaker K^+ channel protein (b) isolated from *Drosophila* has only 616 amino acids; it is similar in sequence and transmembrane structure to each of the four domains in the Na^+ channel protein. [From J. Darnell, H. Lodish, and D. Baltimore, *Molecular Cell Biology*, 2nd edn., Scientific American Books, New York, 1990. Adapted from W. A. Catterall, Structure and function of voltage-sensitive ion channels, *Science* **242**:50–61 (1988). Copyright (1988) American Association for the Advancement of Science.]

Proposed functional description of channel:

Functional regions:-

- *Selectivity filter* – for determining which ions can pass through a channel
- *Gate* – for opening and closing channel
- *Sensor* – for detecting transmembrane potential to control gating

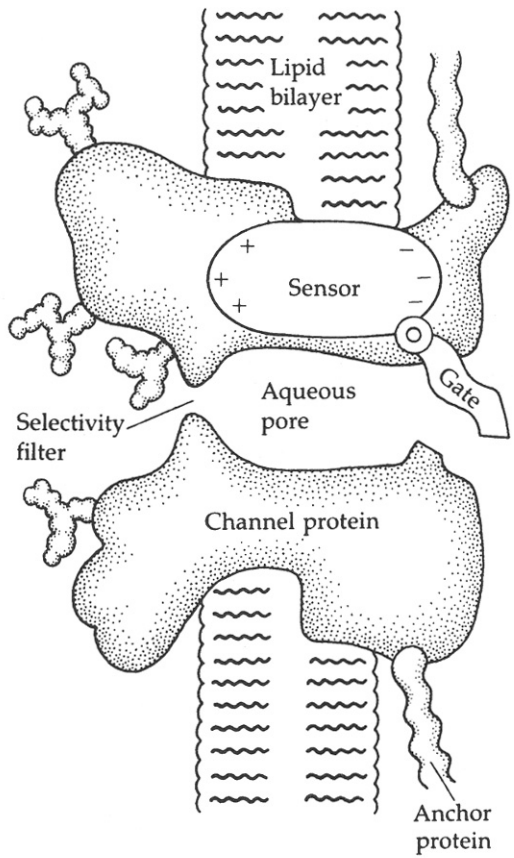


Figure 4.3. Functional description of membrane channel. "The channel is drawn as a transmembrane macromolecule with a hole through the center. The functional regions—selectivity filter, gate, and sensor—are deduced from voltage clamp experiments and are only beginning to be charted by structural studies. We have yet to learn how they actually look." [From B. Hille, *Ionic Channels of Excitable Membranes*, 2nd edn., Sinauer Associates, Sunderland, MA, 1992.]

Channel inactivation:

- In some channels, *inactivation* is thought to be achieved via a voltage-sensitive molecule that can block the channel opening.
- This is referred to as the “ball-and-chain” or “swinging gate” model.

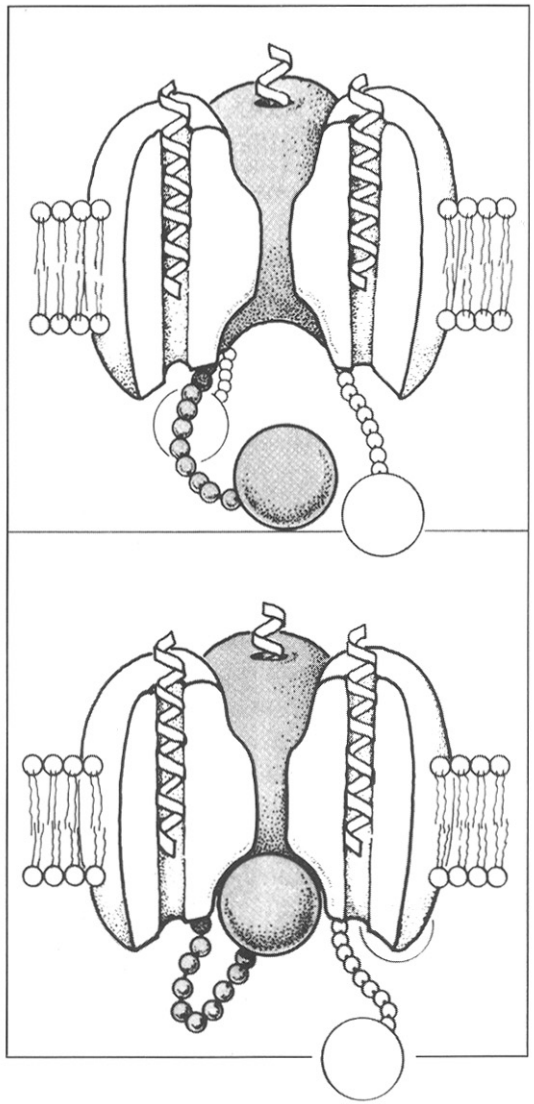
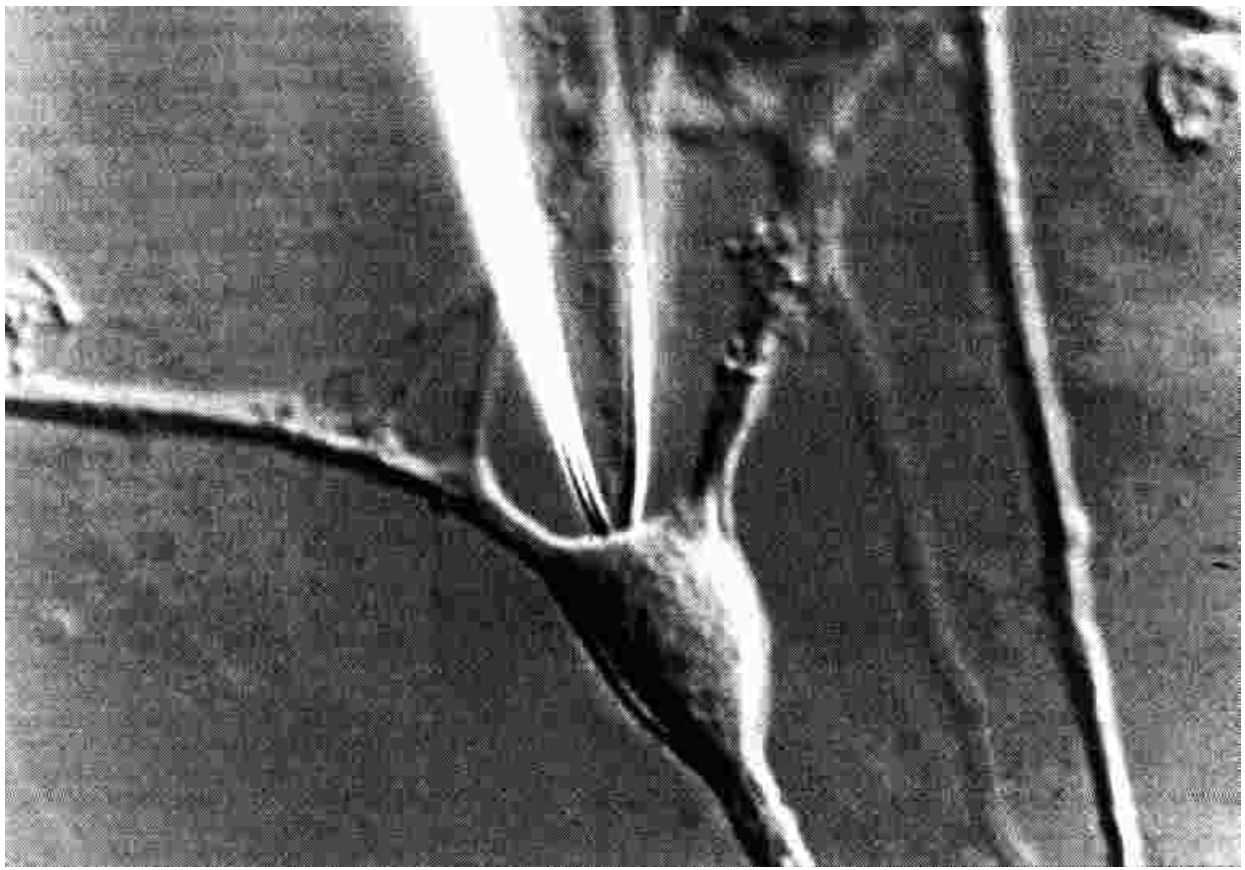


Figure 4.9. A protein ball pops into a pore formed by the bases of four membrane-spanning proteins (one not shown) thereby stopping the flow of potassium ions out of a nerve cell. [Based on T. Hoshi, W. W. Zagotta, and R. W. Aldrich, Biophysical and molecular mechanisms of *Shaker* potassium channel inactivation, *Science* **250**:506–507, 533–538, 568–571 (1990).]

Biophysical methods for measuring channel properties:

Micropipette electrodes are used to measure ionic currents or transmembrane potentials.



Biophysical methods for measuring channel properties (cont.):

- In *patch clamp* recordings, a micropipette forms a tight seal with a membrane patch.
- High leakage resistances are needed to obtain a good signal-to-noise ratio.

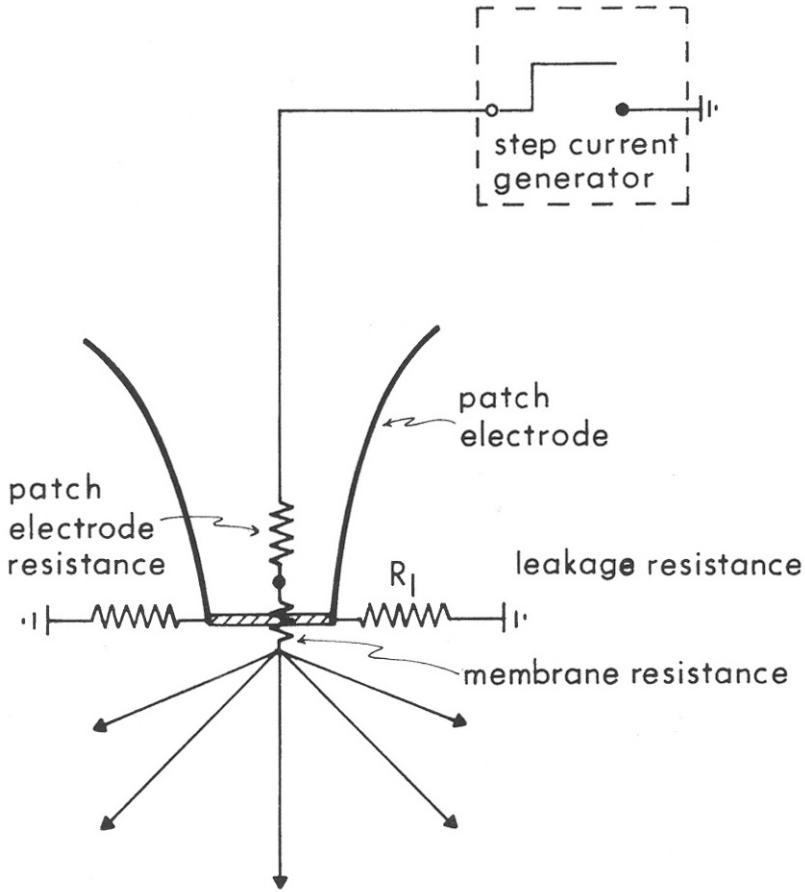


Figure 4.4. Inside-out patch clamp configuration. The desired current path through the cell is challenged by the alternate (leakage) pathway available in the region of electrode–membrane contact. A single open channel is assumed to give a membrane conductance equal to or greater than 20 pS (a resistance of ≤ 50 GΩ). To keep leakage current low (hence minimal loss of signal strength as well as reduced Johnson noise) this resistance should be in the tens of gigaohms; fortunately, patch electrodes with 100 GΩ leakage resistance are currently available.

Four common configurations for patch clamp recordings:

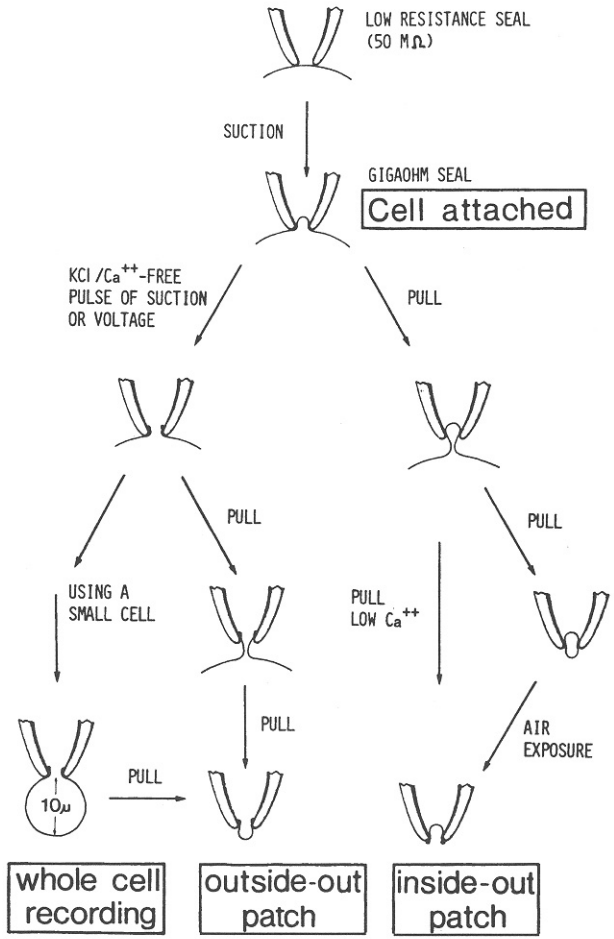


Figure 4.5. Four configurations for patch clamping are described. The clean pipette is pressed against a cell to form a tight seal using light suction, and produces the *cell attached* or *on-cell* configuration. Pulling the pipette away from the cell establishes an *inside-out* patch. Application of a suction pulse disrupts the membrane patch, allowing electrical and diffusional access to the cell interior for *whole-cell* recording. Pulling away from the whole-cell arrangement causes the membrane to re-form into an *outside-out* configuration. [From O. P. Hamill *et al.*, Improved patch clamp techniques for high resolution current recording from cells and cell-free membrane patches, *Pflugers Arch.* **391**:85–100 (1981).]

Single channel recordings:

- Single channels exhibit a *unitary current* when open and zero current when closed.
- Channel opening and closing is stochastic.

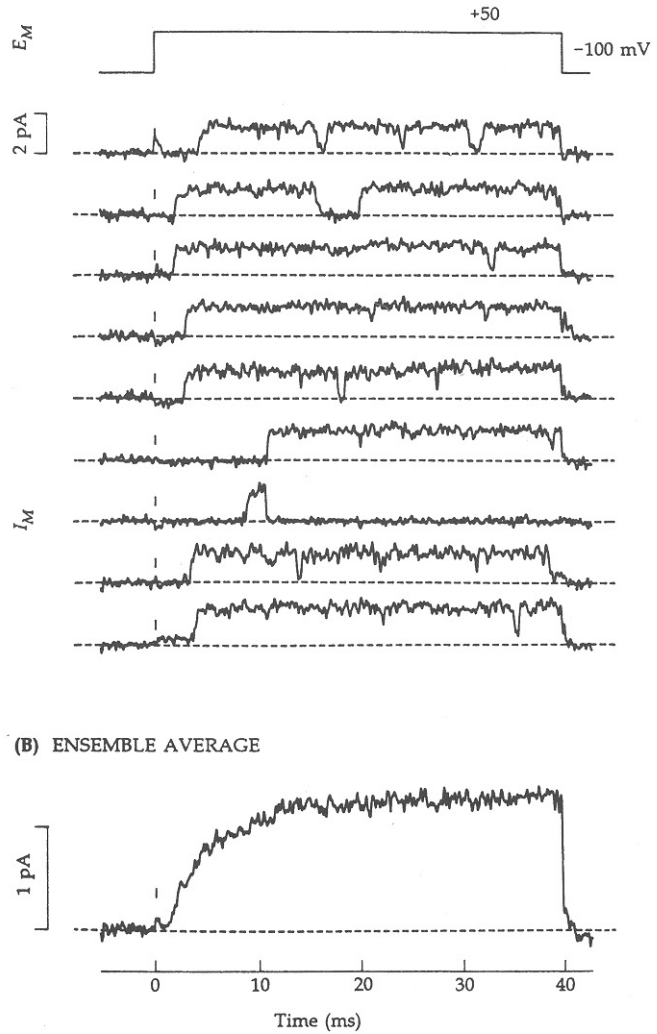


Figure 4.6. Patch clamp recording of unitary K currents in a squid giant axon during voltage steps from –100 to 50 mV. To avoid the overlying Schwann cells the axon was cut open and the patch electrode sealed against the *cytoplasmic* face of the membrane. (A) Nine consecutive trials showing channels of 20 pS conductance filtered at 2 kHz bandwidth. (B) Ensemble mean of 40 repeats; these reveal the expected macroscopic behavior. $T = 20$ °C. [From F. Bezanilla and G. R. Augustine in B. Hille, *Ionic Channels of Excitable Membranes*, 2nd edn., Sinauer Associates, Sunderland, MA, 1992.]

Electrical circuit representation of a single channel:

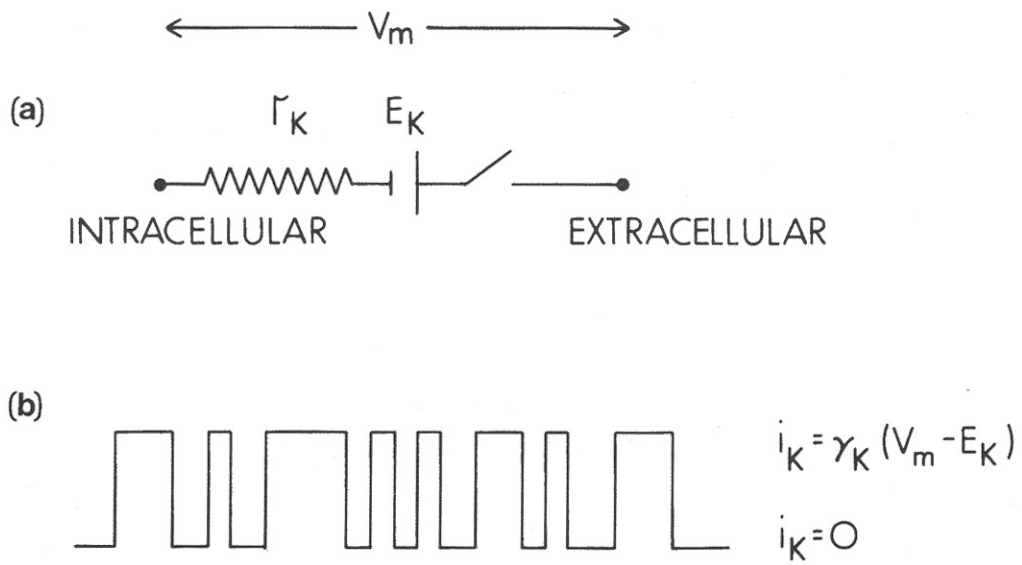


Figure 4.7. (a) Electrical circuit representation for a single (potassium) channel showing fixed resistance r_K, potassium Nernst potential E_K, and the transmembrane potential V_m. The closing and opening of the switch simulates the stochastic opening and closing of the channel gate. (b) Single-channel current corresponding to (a), where $\gamma_K = 1/r_K$. This is an idealization of the recording shown in Fig. 4.6.

Single channel current-voltage relationships:

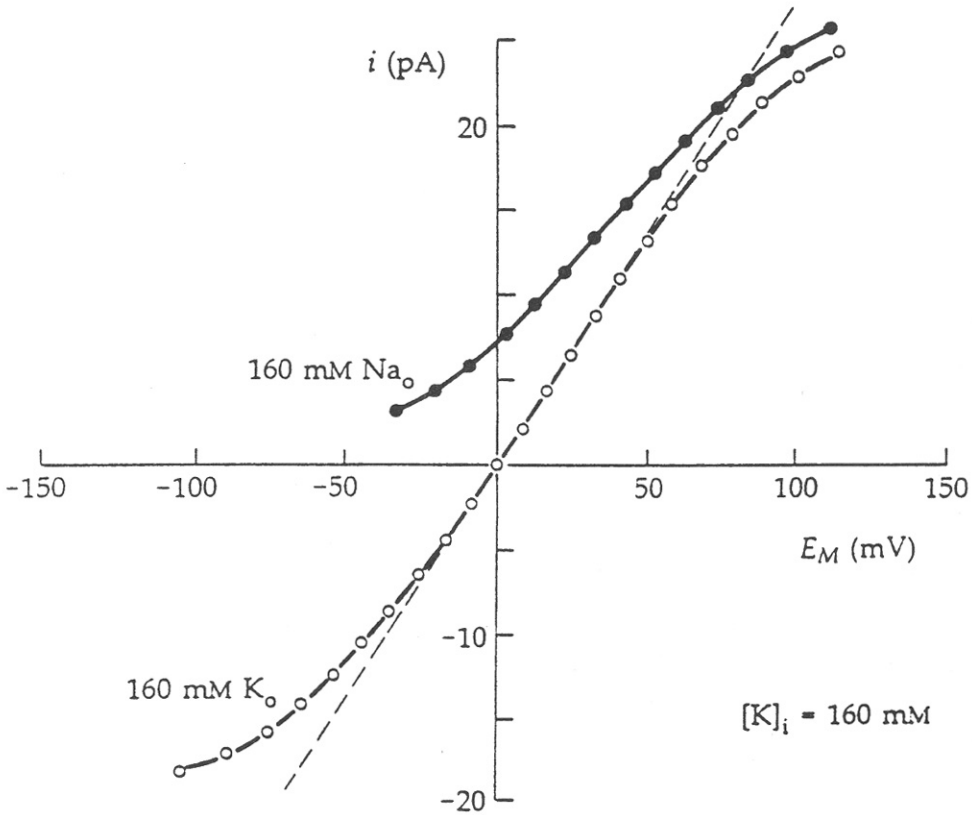


Figure 4.8. Current–voltage relations for a single BK K(Ca) channel of bovine chromaffin cell. The excised outside-out patch was bathed in 160 mM KCl or NaCl and the patch pipette contained 160 m M KCl. In symmetrical K solutions the slope of the dashed line is $\gamma = 265$ pS. $T = 23$ °C. [From B. Hille, *Ionic Channels of Excitable Membranes*, 2nd edn., Sinauer Associates, Sunderland, MA, 1992; based on measurements of G. Yellen, Ionic permeation and blockade in a Ca^{2+}-activated K^+ channels of bovine chromaffin cells, *J. Gen. Physiol.* **84**:157–186 (1984).]

Single channel conductances and channel densities:

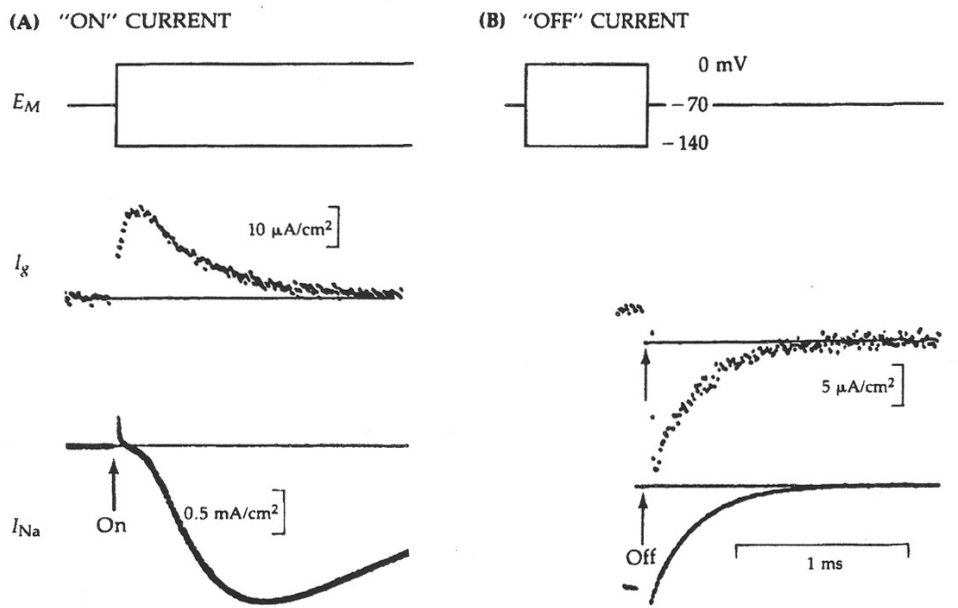
Table 4.1. Conductance and Density of Sodium and Potassium Channels[a]

Preparation	γ (pS)	Channels (number/μm^2)
	Sodium	
Squid giant axon	4	330
Frog node	6–8	400–2000
Rat node	14.5	700
Bovine chromaffin	17	1.5–10
	Potassium	
Squid giant axon	12	30
Frog node	2.7–4.6	570–960
Frog skeletal	15	30
Mammalian BK	130–240	—

[a]From B. Hille, *Ionic Channels of Excitable Membranes*, 2nd edn., Sinauer Associates, Sunderland, MA, 1992, based on data from a number of published measurements.

Channel gating:

Movement of a *charged gating particle* can be measured as a small *gating current*.



(from Johnston and Wu)

Figure 6.22 Gating current (I_g) and I_{Na} recorded by adding responses to symmetrical positive and negative pulses applied to the squid giant axon. I_g was measured in Na^+-free solutions with TTX to block Na^+ channels and internal Cs^+ to block K^+ channels. Since I_g is small, 50 traces had to be averaged in the recording computer to reduce the noise. I_{Na} is measured in normal artificial sea water without TTX. (A) Depolarization from rest elicits an outward "on" I_g that precedes opening of Na^+ channels. (B) Repolarization elicits an inward "off" I_g coinciding with closing of channels (a different axon). (From Hille 1992, adapted from Armstrong and Bezanilla 1974 by copyright permission of the Rockefeller University Press.)