CHAPTER

3

c0015

Ionic gradients, membrane potential and ionic currents

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OUTLINE

p0080 The neuronal plasma membrane delimits the whole neuron, cell body, dendrites, dendritic spines, axon and axon terminals. It is a barrier between the intracellular and extracellular environments. The general structure of the neuronal plasma membrane is similar to that of other plasma membranes. It is made up of proteins inserted in a lipid bilayer, forming as a whole a 'fluid mosaic' (**Figure 3.1**). However, insofar as there are functions that are exclusively neuronal, the neuronal membrane differs from other plasma membranes by the nature, density and spatial distribution of the proteins of which it is composed.

p0085 The presence of a large diversity of transmembrane proteins called *ionic channels* (or simply 'channels') characterizes the neuronal plasma membrane. They allow the passive movement of ions across membranes and thus electrical signaling in the nervous system. Among the ions present in the nervous system fluids, Na^+, K^+, Ca^{2+} and Cl^- ions seem to be responsible for almost all of the action.

3.1 THERE IS AN UNEQUAL DISTRIBUTION OF IONS ACROSS NEURONAL PLASMA MEMBRANE. THE NOTION OF CONCENTRATION GRADIENT

st0025

3.1.1 The plasma membrane separates two media of different ionic composition

st0030

p0090 Regardless of the animal's environment (seawater, freshwater or air), potassium (K^+) ions are the predominant cations in the intracellular fluid and sodium (Na^+) ions are the predominant cations in the extracellular fluid. The main anions of the intracellular fluid are organic molecules (P^-): negatively charged amino acids (glutamate and aspartate), proteins, nucleic acids, phosphates, etc… which have a large molecular weight. In the extracellular fluid, the predominant anions are chloride (Cl^-) ions. A marked difference between cytosolic and extracellular Ca^{2+} concentrations is also observed (**Figure 3.2**).

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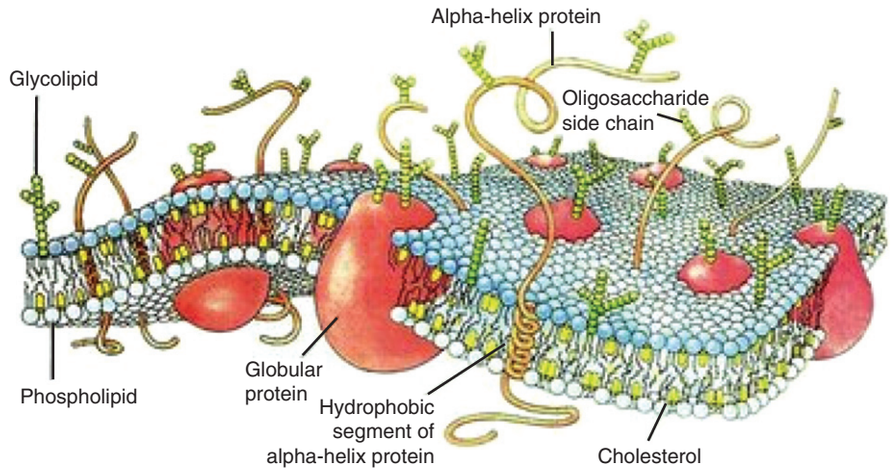
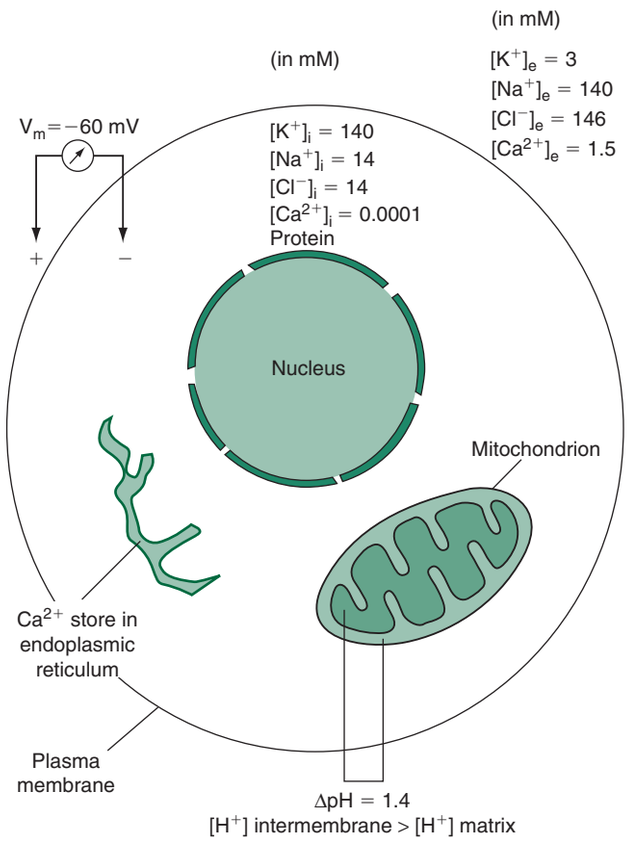


f0010 **FIGURE 3.1** **Fluid mosaic.** Transmembrane proteins and lipids are kept together by non-covalent interactions (ionic and hydrophobic). From dictionary.laborlawtalk.com/Plasma_membrane.



f0015 **FIGURE 3.2** **There is an unequal distribution of ions across neuronal plasma membranes.** Idealized nerve cell (depicted as a sphere) with intra- and extracellular ionic concentrations. Membrane potential is the difference of potential (in mV) between the intracellular and extracellular faces of the plasma membrane.

p0095 Spatial distribution of Ca^{2+} ions inside the cell deserves a more detailed description. Ca^{2+} ions are present in the cytosol as 'free' Ca^{2+} ions at a very low concentration (10^{-7} M) and as bound Ca^{2+} ions (bound to Ca^{2+}-binding proteins). They are also distributed in organelles able to sequester calcium, which include endoplasmic reticulum, calciosome and mitochondria, where they constitute the intracellular Ca^{2+} stores. Free intracellular Ca^{2+} ions present in the cytosol act as second messengers and transduce electrical activity in neurons into biochemical events such as exocytosis. Ca^{2+} ions bound to cytosolic proteins or present in organelle stores are not active Ca^{2+} ions; only 'free' Ca^{2+} ions have a role.

p0100 In spite of the unequal distribution of ions across the plasma membrane, intracellular and extracellular media are neutral ionic solutions: in each medium, the concentration of positive ions is equal to that of negative ions. According to **Figure 3.2**,

$$[Na^+]e + [K^+]e + 2[Ca^{2+}]e = 140 + 3 + (2 \times 1.5) = 146\,\text{mM}$$

$$[Cl^-]e = 146\,\text{mM}$$

$$[Na^+]_i + [K^+]_i + 2[Ca^{2+}]_i = 14 + 140 + 0.0002(2 \times 0.0001) = 154\,\text{mM}$$

p0105 But $[Cl^-]_i = 14$ mM

p0110 In the intracellular compartment, other anions than chloride ions are present and compensate for the positive charges. These anions are HCO_3^-, PO_4^{2-}, amino acids, proteins, nucleic acids, etc. Most of these anions are organic anions that do not cross the membrane.

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3.1.2 The unequal distribution of ions across the neuronal plasma membrane is kept constant by active transport of ions

p0115 A difference of concentration between two compartments is called a '*concentration gradient*'. Measurements of Na^+, K^+, Ca^{2+} and Cl^- concentrations have shown that concentration gradients for ions are constant in the external and cytosolic compartments, at the macroscopic level, during the entire neuronal life.

p0120 At least two hypotheses can explain this constancy:

u0065 • Na^+, K^+, Ca^{2+} and Cl^- ions cannot cross the plasma membrane: plasma membrane is impermeable to these inorganic ions. In that case, concentration gradients need to be established only once in the lifetime.

u0070 • Plasma membrane is permeable to Na^+, K^+, Ca^{2+} and Cl^- ions but there are mechanisms that continuously re-establish the gradients and maintain constant the unequal distribution of ions.

p0135 This has been tested experimentally by measuring ionic fluxes. When proteins are absent from a synthetic lipid bilayer, no movements of ions occur across this purely lipidic membrane. Owing to its central hydrophobic region, the lipid bilayer has a low permeability to hydrophilic substances such as ions, water and polar molecules; i.e. the lipid bilayer is a barrier for the diffusion of ions and most polar molecules.

p0140 The first demonstrations of ionic fluxes across plasma membrane by Hodgkin and Keynes (1955) were based on the use of radioisotopes of K^+ or Na^+ ions. Experiments were conducted on the isolated squid giant axon. When this axon is immersed in a bath containing a control concentration of radioactive $*Na^+$ ($^{24}Na^+$) instead of cold Na^+ ($^{22}Na^+$), $*Na^+$ ions constantly appear in the cytoplasm. This $*Na^+$ influx is not affected by dinitrophenol (DNP), a blocker of ATP synthesis in mitochondria. It does not require energy expenditure. This is *passive* transport. This result is in favor of the second hypothesis and leads to the following question: what are the mechanisms that maintain concentration gradients across neuronal membranes?

p0145 When the reverse experiment is conducted, the isolated squid giant axon is passively loaded with radioactive $*Na^+$ by performing the above experiment, and is then transferred to a bath containing cold Na^+. Measuring the quantity of $*Na^+$ that appears in the bath per unit of time ($d*Na^+/dt$, expressed in counts per minute) allows quantification of the efflux of $*Na^+$ (**Figure 3.3a**). In the presence of dinitrophenol (DNP) this $*Na^+$ efflux quickly diminishes to nearly zero. The process can be started up again by intracellular injection of ATP. Therefore, the $*Na^+$ efflux is *active* transport. The movement of Na^+ from the cytosol to the outside (efflux) can be switched off reversibly by the use of metabolic inhibitors.

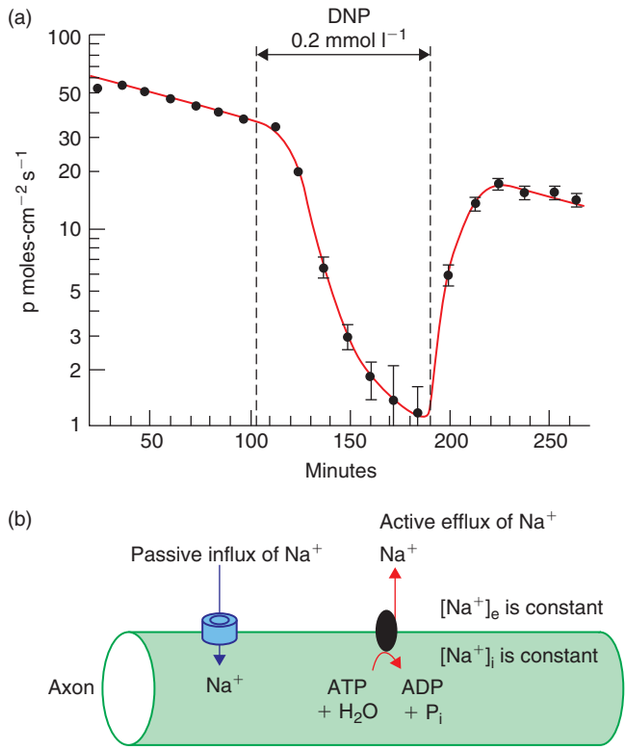

FIGURE 3.3 **Na^+ fluxes through the membrane of giant axons of sepia.** (**a**) Effect of dinitrophenol (DNP) on the outflux of $*Na^+$ as a function of time. The axon is previously loaded with $*Na^+$. At $t = 1$, the axon is transferred in a bath devoid of $*Na^+$. The ordinate (logarithmic) axis is the quantity of $*Na^+$ ions that appear in the bath (that leave the axon) as a function of time. At $t = 100$ min, DNP (0.2 mM) is added to the bath for 90 min. The efflux, which previously decreased linearly with time, is totally blocked after one hour of DNP. This blockade is reversible. (**b**) Passive and active Na^+ fluxes are in opposite directions. Plot (**a**) adapted from Hodgkin AL and Keynes RD (1955) Active transport of cations in giant axons from sepia and loligo. *J. Physiol. (Lond.)* 128, 28-60, with permission. f0020

p0150 This experiment demonstrates that cells maintain their ionic composition in the face of continuous passive exchange of all principal ions by active transport of these ions in the reverse direction. In other words, ionic composition of cytosol and extracellular compartments are maintained at the expense of a continuous basal metabolism that provides energy (ATP) utilized to actively transport ions and thus to compensate for their passive movements (see Appendix 3.1).

st0040

3.1.3 Na^+, K^+, Ca^{2+} and Cl^- ions passively cross the plasma membrane through a particular class of transmembrane proteins – the channels

p0155 Transmembrane proteins span the entire width of the lipid bilayer (see **Figure 3.1**). They have hydrophobic regions containing a high fraction of non-polar amino acids and hydrophilic regions containing a high fraction

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of polar amino acids. Certain hydrophobic regions organize themselves inside the bilayer as transmembrane α-helices while more hydrophilic regions are in contact with the aqueous intracellular and extracellular environments. Interaction energies are very high between hydrophobic regions of the protein and hydrophobic regions of the lipid bilayer, as well as between hydrophilic regions of the protein and the extracellular and intracellular environments. These interactions strongly stabilize transmembrane proteins within the bilayer, thus preventing their extracellular and cytoplasmic regions from flipping back and forth.

p0160 *Ionic channels* have a three-dimensional structure that delimits an aqueous pore through which certain ions can pass. They provide the ions with a passage through the membrane (see Appendix 3.2). Each channel may be regarded as an excitable molecule as it is specifically responsive to a stimulus and can be in at least two different states: closed and open. Channel opening, the switch from the closed to the open state, is tightly controlled (**Table 3.1**) by:

u0075 • a change in the membrane potential – these are voltage-gated channels;

u0080 • the binding of an extracellular ligand, such as a neurotransmitter – these are ligand-gated channels, also called receptor channels or ionotropic receptors;

u0085 • the binding of an intracellular ligand such as Ca^{2+} ions or a cyclic nucleotide;

u0090 • mechanical stimuli such as stretch – these are mechanoreceptors.

The channel's response to its specific stimuli, called gating, is a simple opening or closing of the pore. The pore has the important property of selective permeability, allowing some restricted class of small ions to flow passively down their electrochemical gradients (see Section 3.3). These gated ion fluxes through pores make signals for the nervous system. p0185

3.2 THERE IS A DIFFERENCE OF POTENTIAL BETWEEN THE TWO FACES OF THE MEMBRANE, CALLED MEMBRANE POTENTIAL (V_m) st0045

If a fine-tipped glass pipette (usually called a microelectrode), connected via a suitable amplifier to a recording system such as an oscilloscope, is pushed through the membrane of a living nerve cell to reach its cytoplasm, a potential difference is recorded between the cytoplasm and the extracellular compartment (**Figure 3.2**). In fact, the cell interior shows a negative potential (typically between −60 and −80 mV) with respect to the outside, which is taken as the zero reference potential. Membrane potential (V_m) is by convention the difference between the potential of the internal and external faces of the membrane ($V_m = V_i - V_o$). In the absence of ongoing electrical activity, this negative potential is termed the resting membrane potential (V_{rest}) (**Figure 3.4**). p0190

We have seen above that in the intracellular and extracellular media the concentration of positive ions is equal to that of negative ions. However, there is a very small excess of positive and negative ions accumulated on each side of the membrane. At rest, for example, a small excess of negative ions is accumulated at the internal side of the membrane whereas a small excess of p0195

t0010 **TABLE 3.1** Examples of ionic channels

Channels	Voltage-gated	Ligand-gated			Mechanically gated
Opened by	Depolarization Hyperpolarization	Extracellular ligand	Intracellular ligand		Mechanical stimuli
Localization	Plasma membrane	Plasma membrane	Plasma membrane	Organelle membrane	Plasma membrane
Examples	Na^+ channels	nAChR iGluR	G protein-gated channels	IP_3-gated Ca^{2+} channel	Stretch-activated channels
	Ca^{2+} channels	$5\text{-}HT_3$	Ca^{2+}-gated channels	Ca^{2+}-gated Ca^{2+} channel	
	K^+ channels Cationic channels	$GABA_A$ GlyR	CNG channels ATP-gated channels		
Closed by	Inactivation Repolarization	Desensitization Ligand recapture or degradation			Adaptation End of stimulus
Roles	Na^+ and Ca^{2+}-dependent action potentials $[Ca^{2+}]_i$ increase	EPSP IPSP $[Ca^{2+}]_i$ increase	EPSP IPSP Action potential repolarization	$[Ca^{2+}]_i$ increase	Receptor potential

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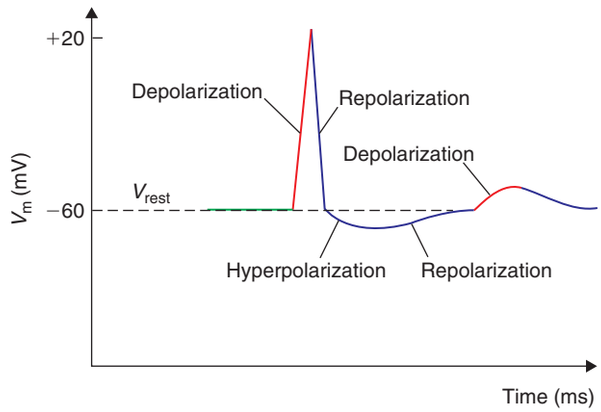


f0025 **FIGURE 3.4 Variations of the membrane potential of neurons (V_m).** When the membrane potential is less negative than resting membrane potential (V_{rest}), the membrane is said to be depolarized. In contrast, when the membrane potential is more negative then V_{rest}, the membrane is said to be hyperpolarized. When the membrane varies from a depolarized or hyperpolarized value back to rest, the membrane repolarizes.

positive ions is accumulated at the external side of the membrane (see Section 3.5). This creates a difference of potential between the two faces of the membrane: the external side is more positive than the internal side, which makes $V_m = V_i - V_o$, negative.

p0200 What is particular to membrane of neurons (and of all excitable cells) is that V_m varies (**Figure 3.4**). It can be more negative or hyperpolarized or less negative (depolarized) or even positive (also depolarized, the internal face is positive compared to the external face). At rest, V_m is in the range $-80/-50$ mV depending on the neuronal type. But when neurons are active, V_m varies between the extreme values −90 mV and +30 mV. Since nerve cells communicate through rapid (milliseconds; ms) or slow (seconds; s) changes in their membrane potential, it is important to understand V_{rest} first.

st0050

3.3 CONCENTRATION GRADIENTS AND MEMBRANE POTENTIAL DETERMINE THE DIRECTION OF THE PASSIVE MOVEMENTS OF IONS THROUGH IONIC CHANNELS: THE ELECTROCHEMICAL GRADIENT

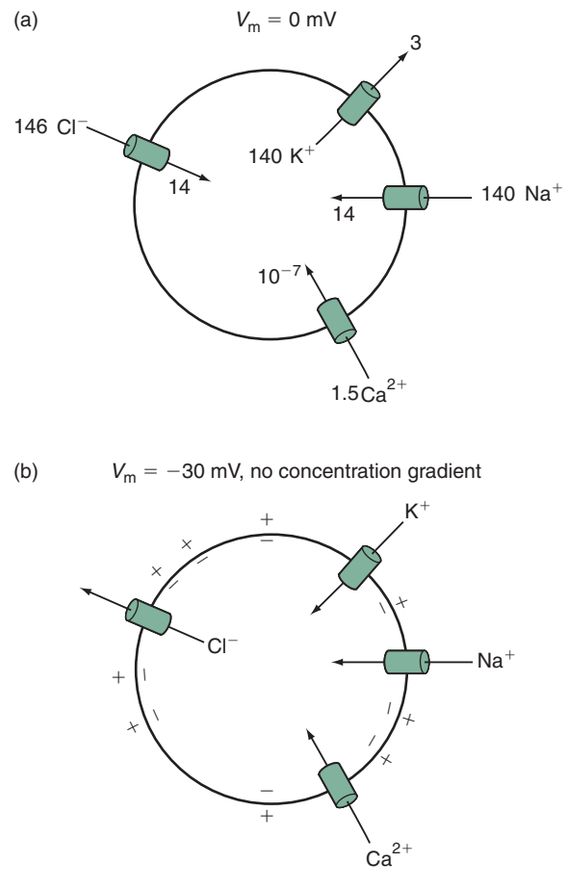
p0205 To predict the direction of the passive diffusion of ions through an open channel, both the concentration gradient of the ion and the membrane potential have to be known. The *resultant* of these two forces is called the electrochemical gradient. To understand what the electrochemical gradient is for a particular ion, the concentration gradient and the electrical gradient will first be explained separately.

st0055

3.3.1 Ions passively diffuse down their concentration gradient

p0210 The concentration gradient of a particular ion is the difference of concentration of this ion between the two sides of the plasma membrane. Ions passively move through open channels from the medium where their concentration is high to the medium where their concentration is lower. Suppose that membrane potential is null ($V_m = 0$ mV), there is no difference of potential between the two faces of the membrane, so ions will diffuse according to their concentration gradient only (**Figure 3.5a**). Since the extracellular concentrations of Na^+, Ca^{2+} and Cl^- are higher than the respective intracellular ones, these ions will diffuse passively towards the intracellular medium (when Na^+, Ca^{2+} or Cl^- permeable channels are open) as a result of their concentration gradient. In contrast, K^+ will move from the intracellular medium to the extracellular one (when K^+ permeable channels are open).

The force that makes ions move down their concentration gradient is *constant* for a given ion since it depends on the difference of concentration of this ion, which is itself continuously controlled to a constant value by active



f0030 **FIGURE 3.5 Passive diffusion of ions.** Passive diffusion of ions according to (**a**) their concentration gradient only, or (**b**) to membrane potential (electrical gradient) only ($V_m = -30$ mV).

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p0215 transport (pumps and transporters). However, this is not always true; during intense neuronal activity, concentration of ions may change (K^+ concentration in particular) owing to the small volume of the external medium in physiological conditions. At the microscopic level, this is not true also; intracellular Ca^{2+} concentration, for example, can increase locally by a factor of between 100 and 1000 but stay stable in the entire cytosol. However, these increases of ion concentration do not change the direction of the concentration gradient for this ion since ionic gradients cannot reverse by themselves.

st0060 ## 3.3.2 Ions passively diffuse according to membrane potential

p0220 Membrane potential is a potential gradient that forces ions to passively move in one direction: positive ions are attracted by the 'negative' side of the membrane and negative ions by the 'positive' one. If we suppose that there is no concentration gradient for any ions (there is the same concentration of each ion in the extracellular and intracellular media), ions will diffuse according to membrane potential only: at a membrane potential $V_m = -30$ mV (**Figure 3.5b**), positively charged ions, the cations Na^+, Ca^{2+} and K^+, will move from the extracellular medium to the intracellular one according to membrane potential. In contrast, anions (Cl^-) will move from the intracellular medium to the extracellular one.

st0065 ## 3.3.3 In physiological conditions, ions passively diffuse according to the electrochemical gradient

p0225 In physiological conditions, both the concentration gradient and membrane potential determine the direction and amplitude of ion diffusion through an open channel. Since concentration gradient is constant for each ion, the direction and amplitude of diffusion varies with membrane potential. When comparing **Figure 3.5a** and **b** it appears that at a membrane potential of −30 mV, concentration gradient and membrane potential drive Na^+ and Ca^{2+} ions in the same direction, toward the intracellular medium, whereas they drive K^+ and Cl^- in reverse directions. The resultant of these two forces, concentration and potential gradients, is the electrochemical gradient. To know how to express the electrochemical gradient, the equilibrium potential must first be explained.

st0070 ### *The equilibrium potential for a given ion, E_{ion}*

p0230 All systems are moving toward equilibrium. The value of membrane potential where the concentration force that tends to move a particular ion in one direction is exactly balanced by the electrical force that tends to move the same ion in the reverse direction is called the 'equilibrium potential' of the ion (E_{ion}) or the reversal potential of the ion E_{rev}. The equilibrium potential for a particular ion is the value of V_m for which the net flux of this ion (f_{net}) through an open channel is null: when $V_m = E_{ion}$, $f_{net} = 0$ mol s^{-1}.

p0235 E_{ion} can be calculated using the Nernst equation (see Appendix 3.3):

$$E_{ion} = (RT/zF)\ln([ion]_e/[ion]_i),$$

where R is the constant of an ideal gas (8.314 VCK^{-1} mol^{-1}); T is the absolute temperature in kelvin (273.16 + the temperature in °C); F is the Faraday constant (96 500 C mol^{-1}); z is the valence of the ion; and [ion] is the concentration of the ion in the extracellular (e) or intracellular (i) medium. This gives:

$$E_{ion} = (58/z)\log_{10}([ion]_e/[ion]_i), \quad (1)$$

p0240 From the equation and concentrations of **Figure 3.2**, the equilibrium potentials for each ion can be calculated:

$$E_{Na} = (58/1)\log_{10}(140/14) = +58\,\text{mV}$$

$$E_K = (58/1)\log_{10}(3/140) = -97\,\text{mV}$$

$$E_{Ca} = (58/2)\log_{10}(1.5/10^{-4}) = +121\,\text{mV}$$

$$E_{Cl} = (58/-1)\log_{10}(146/14) = -59\,\text{mV}.$$

p0245 These equations have the following meanings. If the channels open in a membrane where K^+ channels are the only channels open, the efflux of K^+ ions will hyperpolarize the membrane until $V_m = E_K = -97$ mV, a potential at which the net flux of K^+ is null since K^+ ions have exactly the same tendency to diffuse towards the intracellular medium according to their concentration gradient than to move in the reverse direction according to membrane potential. At that potential, the efflux of K^+ will be exactly compensated by the influx of K^+ and the membrane potential will stay stable at $V_m = E_K$ as long as K^+ channels stay open. Now, if only Na^+ channels are open, the membrane potential will move toward $V_m = +58$ mV, the potential at which the net flux of Na^+ is null. Similarly, when $V_m = E_{Cl} = -59$ mV, Cl^- ions have the same tendency to move down their concentration gradient than to move in the reverse direction according to membrane potential, the net flux of Cl^- is null. In contrast, when V_m is different from E_{Cl}, the net flux of Cl^- is not null. This holds true for all the other ions: when V_m is different from E_{ion} there is a net flux of this ion.

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st0075 ***The electrochemical gradient***

p0250 We have seen that when $V_m = E_{ion}$ (i.e. $V_m - E_{ion} = 0$), there is no diffusion of this particular ion ($f_{net} = 0$). In contrast, when V_m is different from E_{ion} there is a passive diffusion of this ion through an open channel. The difference ($V_m - E_{ion}$) is called the electrochemical gradient. It is the force that makes the ions move through an open channel.

st0080

3.4 THE PASSIVE DIFFUSION OF IONS THROUGH AN OPEN CHANNEL CREATES A CURRENT

p0255 To know the direction of passive diffusion of a particular ion and how many of these ions diffuse per unit of time, the direction and intensity of the net flux of ions (number of moles per second) through an open channel have to be measured. Usually the net flux (f_{net}) is not measured; the electrical counterpart of this net flux, the ionic current, is measured instead.

p0260 Passive diffusion of ions through an open channel is a movement of charges through a resistance (resistance here is a measure of the difficulty of ions moving through the channel pore). Movement of charges through a resistance is a current. Through a single channel the current is called 'single-channel current' or 'unitary current', i_{ion}. The relation between f_{net} and i_{ion} is:

$$i_{ion} = f_{net}\, zF$$

p0265 The amplitude of i_{ion} is expressed in ampères (A) which are coulombs per seconds (C.s^{-1}). F is the Faraday constant (96 500 C.moles^{-1}); z is the valence of the ion (+1 for Na^+ and K^+, −1 for Cl^-, +2 for Ca^{2+}); and f_{net} is the net flux of the ion in mol.s^{-1}.

p0270 In general, currents are expressed following Ohm's Law: $U = RI$, where I is the current through a resistance R and U is the difference of potential between the two ends of the resistance. For currents carried by ions (and not by electrons as in copper wires), I is called i_{ion}, the current that passes through the resistance of the channel pore which has a resistance R (called r_{ion}). But what is U in biological systems? U is the force that makes ions move in a particular direction; it is the electrochemical gradient for the considered ion and is also called the driving force: $U = V_m - E_{ion}$ (**Figure 3.6**).

st0085 ***Unitary current, i_{ion}***

p0275 According to Ohm's Law, the current i_{ion} through a single channel is derived from

$$(V_m - E_{ion}) = r_{ion} \cdot i_{ion}$$

p0280 So:

$$i_{ion} = (1 / r_{ion})(V_m - E_{ion}) = \gamma_{ion}(V_m - E_{ion})$$

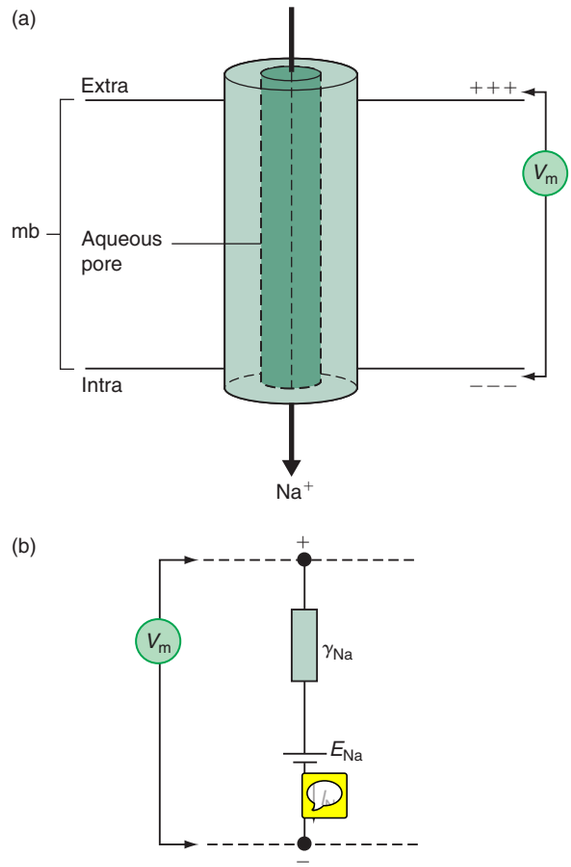

FIGURE 3.6 **The Na^+ channel.** (**a**) Schematic, and (**b**) its electrical equivalent. f0035

γ_{ion} is the reciprocal of resistance; it is called the *conductance* of the channel, or unitary conductance (**Figure 3.6**). It is a measure of the ease of flow of ions (flow of current) through the channel pore. Whereas resistance is expressed in ohms (Ω), conductance is expressed in siemens (S). By convention i_{ion} is negative when it represents an inward flux of positive charges (cations) and i_{ion} is positive when it represents an outward flux of positive charges (**Figure 3.5c**). It is generally of the order of pico-ampères (1 pA = 10^{-12}A). At physiological concentrations, γ_{ion} varies between 10 and 150 pico-siemens (pS), according to the channel type. p0285

Total current, I_{ion} st0090

In physiological conditions, several channels of the same type are open at the same time in the neuronal membrane. Suppose that only one type of channel is open in the membrane, for example Na^+ channels, the total current I_{Na} that crosses the membrane at time t is the sum of the unitary currents i_{Na} at time t: p0290

$$I_{Na} = Np_o\, i_{Na}$$

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where N is the number of Na^+ channels present in the membrane; p_o is the probability of Na^+ channels being open at time t (Np_o is therefore the number of open Na^+ channels in the membrane at time t); and i_{Na} is the unitary Na^+ current. More generally:

$$I_{ion} = Np_o\, i_{ion}$$

p0295 By analogy, the total conductance of the membrane for a particular ion is:

$$G_{ion} = Np_o\, \gamma_{ion}$$

and from $i_{ion} = \gamma_{ion}(V_m - E_{ion})$ above:

$$I_{ion} = G_{ion}(V_m - E_{ion})$$

p0300 I_{ion} and i_{ion} can be measured experimentally. The latter is the current measured from a patch of membrane where only one channel of a particular type is present. I_{ion} is the current measured from a whole cell membrane where N channels of the same type are present.

st0095 ***Roles of ionic currents***

p0305 Ionic currents have two main functions:

u0095 • Ionic currents change the membrane potential: either they depolarize the membrane or repolarize it or hyperpolarize it, depending on the charge carrier. These terms are in reference to resting potential (**Figure 3.4**). Changes of membrane potential are signals. A depolarization can be an action potential (see Chapters 4 and 5) or a postsynaptic excitatory potential (EPSP; see Chapters 8 and 10). A hyperpolarization can be a postsynaptic inhibitory potential (IPSP; see Chapter 9). These changes of membrane potential are essential to neuronal communication.

u0100 • Ionic currents increase the concentration of a particular ion in the intracellular medium. Calcium current, for example, is always inward. It transiently and locally increases the intracellular concentration of Ca^{2+} ions and contributes to the triggering of Ca^{2+}-dependent events such as secretion or contraction.

st0100

3.5 A PARTICULAR MEMBRANE POTENTIAL, THE RESTING MEMBRANE POTENTIAL V_{rest}

p0320 In the absence of ongoing electrical activity (when the neuron is not excited or inhibited by the activation of its afferents), its membrane potential is termed the resting membrane potential (V_{rest}). For some neurons, V_{rest} is stable (silent neurons) for others it is not (pacemaker neurons for example). In this section, we will consider stable V_{rest} only. To understand unstable V_{rest} many different channels must be known that are explained later in the book (see Chapter 14).

3.5.1 When most of the channels open at rest are K^+ channels, V_{rest} is close to E_K

st0105

p0325 It was Julius Bernstein (1902) who pioneered the theory of V_{rest} as due to selective permeability of the membrane to one ionic species only and that nerve excitation developed when such selectivity was transiently lost. According to this theory, under resting conditions, the cell membrane permeability is minimal to Na^+, Cl^- and Ca^{2+} while it is high to K^+. What is the membrane potential of a membrane permeable to K^+ ions only? This condition can be tested experimentally by measuring ionic fluxes with radioactive tracers through a plasma membrane where K^+ channels are the only open channels. K^+ moves outwards following its concentration gradient (the intracellular concentration of K^+ is around 50 times higher than the extracellular one): positive charges are thus subtracted from the intracellular medium and there is an accumulation of negative charges at the intracellular side of the membrane and positive charges at the external side of the membrane. These positive charges will oppose further outward movements of K^+ until an equilibrium is reached when the concentration gradient for K^+ cancels the drive exerted by the electrical gradient. This is by definition the *equilibrium potential* E_K. Hence, at $V_m = E_K$, although K^+ keeps moving in and out of the cell, there is no net change in its concentration across the membrane (**Figure 3.7**). In a physiological situation, the exact value of E_K is unknown since the exact $[K^+]_i$ is unknown. When $V_{rest} = -80/-70$ mV, though it seems close to E_K it may not be equal to E_K.

p0330 A way to test whether $V_{rest} = E_K$ is the following. Inspection of the Nernst equation applied to K^+ indicates that a 10-fold change in the concentration ratio should alter the membrane potential of a neuron by 58 mV. This relation can be tested in experiments in which the extracellular concentration of this ion is altered and the resulting membrane potential measured with a sharp or patch microelectrode. A semilog plot of the extracellular K^+ concentration (abscissa) against the membrane potential (ordinate) should thus have a slope of 58 mV per 10-fold change in K^+ (**Figure 3.8**); this condition is rarely encountered in neurons but it seems to be more common for glial cells (which sometimes are termed K^+ electrodes because their membrane potential is linearly dependent on K^+). In the case of neurons, non-linearity of this plot is frequently seen, particularly at low levels of extracellular K^+. These observations confirm that K^+ is a very important ion for setting the value of neuronal V_{rest} but that other ions must also play a significant role.

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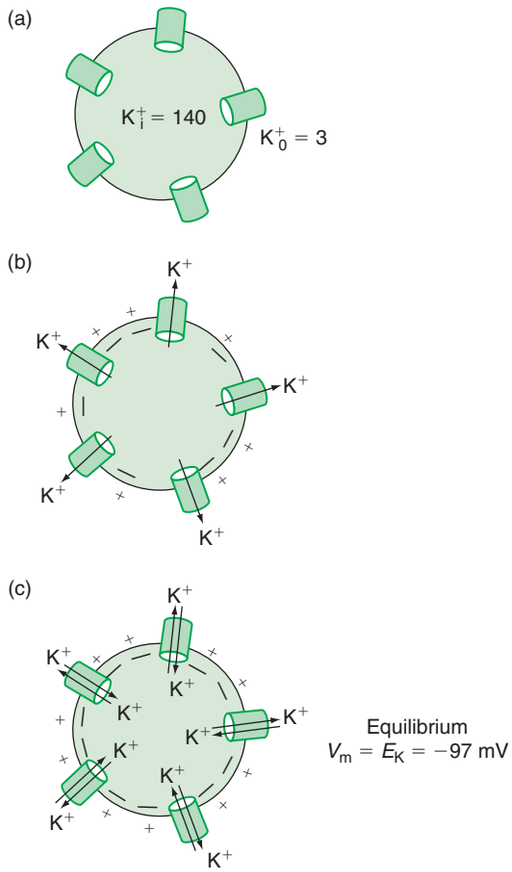
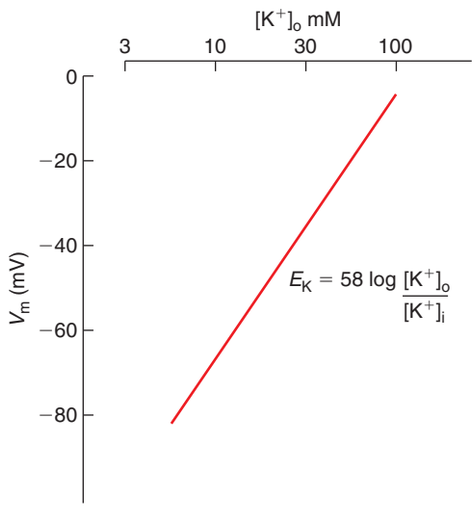


f0040 **FIGURE 3.7 Establishment of V_{rest} in a cell where most of the channels open are K^+ channels.** Suppose that at $t = 0$ and cell potential $V_m = 0$ mV (**a**), K^+ ions will move outwards due to their concentration gradient (**b**). Loss of intracellular K^+ induces a negative potential (V_m) as $V_m = E_K$ (**c**).



f0045 **FIGURE 3.8 Theoretical diagram of E_K versus the external concentration of K^+ ions ($[K^+]o$).** $E_K = (RT/zF)\ 2.3 \times \log([K^+]_o/[K^+]_i)$.

3.5.2 In central neurons, K^+, Cl^- and Na^+ ion movements participate in resting membrane potential and V_{rest} is different from E_K: the Goldman–Hodgkin–Katz equation

st0110

Aside from K^+, which ions play a role in V_{rest}? Since the intracellular concentration of Na^+ is not negligible, this implies that this ionic species can accumulate inside the cytoplasm, presumably because of its rather positive E_{Na} (+58 mV) versus a very negative V_{rest} creates an electrochemical gradient extremely favorable to Na^+ entry. Equally, the asymmetric distribution of Cl^- suggests its possible role in determining V_{rest}. In order to take into account various ionic species it is useful to introduce what is commonly called the *Goldman–Hodgkin–Katz equation* (GHK), derived from the Nernst equation and named after the three physiologists responsible for its derivation:

p0335

$$V_{rest} = 58 \log \times \frac{p_K[K^+]_o + p_{Na}[Na^+]_o + p_{Cl}[Cl^-]_i}{p_K[K^+]_i + p_{Na}[Na^+]_i + p_{Cl}[Cl^-]_o} \qquad (2)$$

where p is the permeability coefficient (cm s^{-1}) for each ionic species. The relative contribution of each ion species to the resting voltage is weighted by that ion's permeability.

Note that if the resting permeability to Na^+ and Cl^- is very low, the GHK equation closely resembles the Nernst equation for K^+.

p0340

In applying the GHK equation to nerve cells, the following assumptions must be made:

p0345

- The voltage gradient across the membrane is uniform in the sense that it changes linearly within the membrane. This assumption has led to the GHK equation being called the *constant field equation*. u0105
- The overall net current flow across the membrane is zero as the currents generated by individual ionic species are balanced out. u0110
- The membrane is in a steady state since there is no time-dependent change in ionic flux or channel density. This is obviously not applicable to non-steady state conditions of rapidly changing membrane potential as produced when a nerve cell fires action potentials. u0115
- Any role of active transport mechanisms is ignored. u0120
- The ionic species are monovalent cations or anions which do not interact among themselves or with water molecules. The first point does not hold true if there is a measurable permeability to divalent cations such as Ca^{2+}. Furthermore, it has been reported that ions can interact among themselves within the same channel. u0125

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u0130 • The role of membrane surface charges is ignored. This is a relatively major limitation because the cell membrane contains negative charges on its inner and outer layers (amino acid residues of membrane proteins which are typically negatively charged). The electric field generated by these charges is able to influence the kinetic properties of ionic channels (gating, activation and inactivation). Adding divalent cations such as Ca^{2+} or Mg^{2+} leads to screening of these charges and consequent changes in channel properties.

u0135 • The mobility of each ionic species and its diffusion coefficient (D) within the membrane of thickness (δ) is constant.

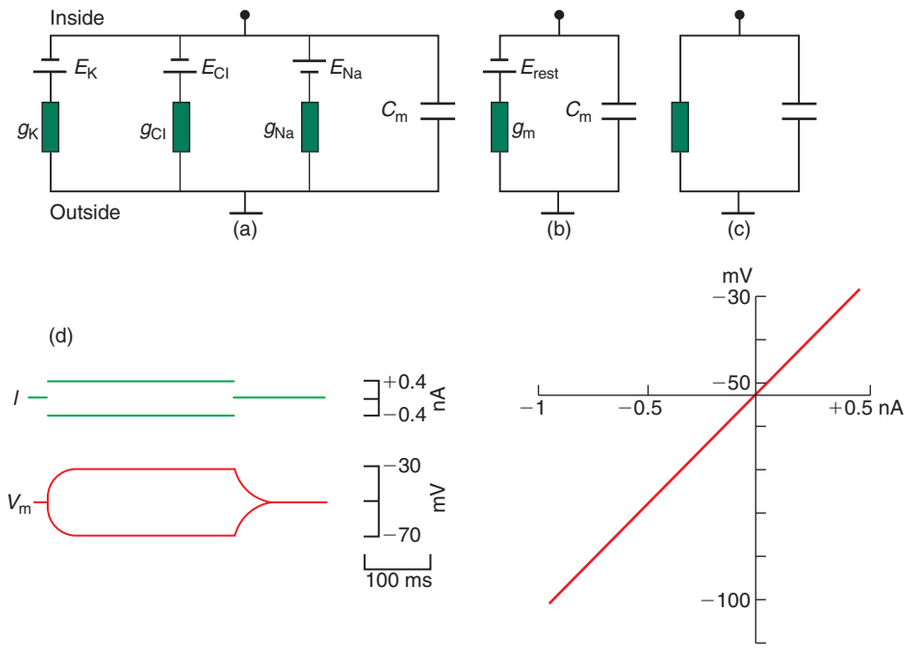
u0140 • The ions do not bind to specific sites in the membrane and their concentration (C) can be expressed by a linear partition coefficient ($\beta = C_{membrane}/C_{solution}$). However, there is evidence that ions can bind to sites inside channels and influence channel kinetics.

u0145 • The ionic activities (a) can be replaced by their concentrations.

3.6 A SIMPLE EQUIVALENT ELECTRICAL CIRCUIT FOR THE MEMBRANE AT REST st0115

Since the plasma membrane does not allow the passage of all the ions at all times, it can be equated to an insulator separating two electrically conductive media (intracellular and extracellular electrolytes): it thus plays the role of a dielectric in a *capacitor* and it can be assigned an average capacity (C_m) value of 1 $\mu F\ cm^{-2}$. p0395

In **Figure 3.9b**, instead of three parallel current sources for K^+, Na^+ and Cl^-, we have lumped them together into only one source with driving (electromotive) force E equal to V_{rest} and an inward conductance g_m equal to the sum of the specific ionic (channel) conductances $g_K + g_{Na} + g_{Cl}$. One may consider, instead of the absolute value of membrane potential, only its deviation from V_{rest}. In this case, the equivalent electromotive force becomes equal to zero and the equivalent scheme of the cell membrane simplifies to an *RC*-circuit (**Figure 3.9c**). If one includes more channel types, then the notion of resting current still holds true. The equivalent scheme of **Figure 3.9c** is p0400



f0050 **FIGURE 3.9 Simplified equivalent scheme to account for membrane electrical characteristics near the resting potential and ohmic behavior of the membrane potential around the resting potential.** (**a**) Three main ionic current sources. Note: E_K and E_{Cl} are negative while E_{Na} is positive. (**b**) An equivalent current source for the resting potential. (**c**) Electrical scheme for below-threshold potential changes (passive de- and hyperpolarizations) relative to the resting potential. Battery symbols indicate electromotive forces, boxes represent conductances and parallel plates indicate membrane capacitors. (**d**) From top to bottom: Time-dependent responses to ±0.4 nA current injected for 300 ms; left: upper traces, current I; middle traces, membrane potential changes V_m; right: membrane potential at the end of the current pulse (i.e. at 300 ms) plotted against current intensity. From Adams PR, Brown DA, Constanti A (1982) M-currents and other potassium currents in bullfrog sympathetic neurones. *J. Physiol. (Lond.)* 330, 537-572, with permission.

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applicable only to depolarizations and hyperpolarizations characterized by linear (ohmic) current–voltage relations (**Figure 3.9d**). In standard excitable cells it means that these potential changes from V_{rest} are not activating voltage-gated currents; e.g. they are below the threshold for spike generation.

st0120

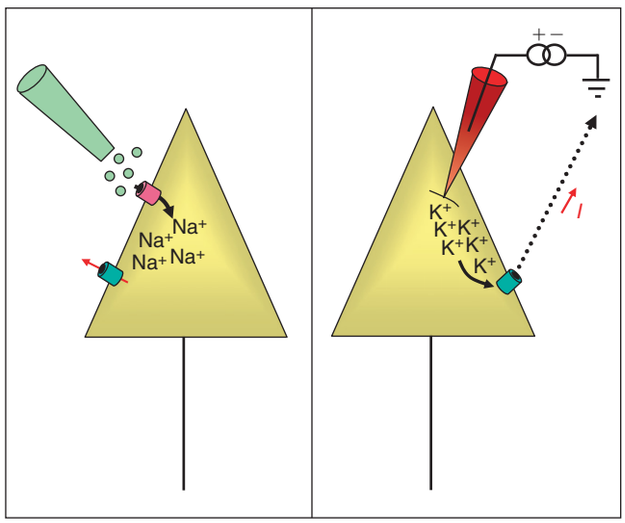
3.7 HOW TO EXPERIMENTALLY CHANGE V_{rest}

st0125

3.7.1 How to experimentally depolarize a neuronal membrane

p0405 The aim of the experiment is to lower the difference of potential between the two faces of the membrane and even to reverse it. There are at least three main ways of depolarizing a membrane: (a) by increasing the K^+ concentration in the external medium, (b) by applying a drug that opens cationic channels or (c) by injecting a positive current inside the neuron (**Figure 3.10**).

p0410 An *in vitro* preparation such as a neuronal culture or a brain slice is bathed in an extracellular solution of an ionic composition close to that of the extracellular medium. A recording electrode is implanted in a neuronal cell body. At rest the membrane potential is close to −70 mV. When the extracellular solution is changed to one containing a higher concentration of K^+ ions (30 mM instead of 3 mM) and a lower concentration of Na^+ ions (113 mM instead of 140 mM) to keep constant the extracellular concentration of positive ions, a depolarization is recorded. Since at rest most of the channels open are K^+ channels, V_m tends toward E_K which is now equal to −38 mV (E_K = 58 log 30/140) instead of −97 mV. The membrane depolarizes because E_K is more depolarized than V_{rest}.



f0055 **FIGURE 3.10** **Vm is depolarized by applying:** (**a**) a drug in the extracellular medium that opens Na^+ channels(veratridine) or (**b**) a positive current via an intracellular electrode.

p0415 In the same preparation bathed in control extracellular medium, veratridine is applied by pressure via a pipette located close to the recorded neuron. Veratridine induces a depolarization of the recorded membrane (**Figure 3.10a**). As this drug opens Na^+ channels, Na^+ ions enter the cell and create an inward current of positive charges. The electrical circuit is closed because + charges can go out of the cell via the K^+ channels open at rest. Since Na^+ channels now represent the major population of open channels, V_m tends toward E_{Na} (+58 mV) and the membrane depolarizes as long as veratridine is applied.

p0420 If now a positive current is applied through the recording pipette which contains a KCl solution, K^+ ions are expelled from the pipette. They create a current of positive charges that depolarizes the membrane (**Figure 3.10b**). The electrical circuit is closed because K^+ ions can go through the membrane via the K^+ channels open at rest. A depolarizing current pulse is a positive current injected via an intracellular electrode. One part of the stimulating current is used to load the capacity C_m of the neuronal membrane and the other part passes through the ion channels:

$$I_{stimulus} = C_m \, dV/dt + I_{ion}$$

$$dV/dt = [-I_{ion} + I_{stimulus}]/C_m$$

p0425 A positive stimulating current applied at the inside of a neuron (cell body, dendrite, axon) will cause a depolarization of V_m according to the above equation. Inversely, a negative current will hyperpolarize the membrane (see below). Once the membrane capacity is loaded (steady state) the injected current equals the current passing through the membrane via open channels.

p0430 In the case of a silver electrode inside the pipette, as a coat of AgCl is deposited on the silver metal, it provides a store of Ag^+ and Cl^- ions and mediates between electronic conduction in the metal ($Ag^+ + e^- \leftrightharpoons Ag$) and ionic current owing that Cl^- exchanges between precipitate (AgCl) and solution.

st0130

3.7.2 How to experimentally hyperpolarize a neuronal membrane

p0435 The aim of the experiment is to increase the difference of potential between the two faces of the membrane. There are at least two main ways of hyperpolarizing a membrane: (a) by applying a drug that opens K^+ channels or (b) by injecting a negative current inside the neuron.

p0440 An *in vitro* preparation such as a neuronal culture or a brain slice is bathed in a physiological saline of an ionic

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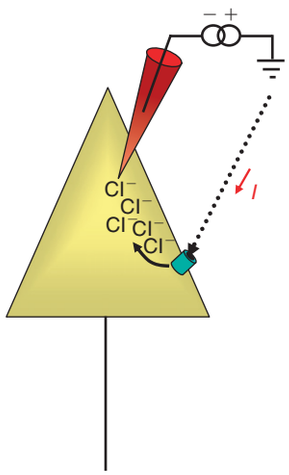

f0060 **FIGURE 3.11** **Vm is hyperpolarized by applying a negative current via an intracellular electrode.**

composition close to that of the extracellular medium. A recording electrode is implanted in a neuronal cell body. A peptide that opens K^+ channels is applied by pressure via a pipette located close to the recorded neuron. This induces a hyperpolarization of the membrane, due the outward flux of K^+ ions. As this drug opens K^+ channels (via metabotropic receptors such as $GABA_B$ receptors, see Chapter 11), K^+ ions exit the cell and create an outward current of positive charges. The electrical circuit is closed because ions can enter the membrane via the channels open at rest. As K^+ channels now represent the major population of open channels, V_m tends toward E_K (−97 mV) and the membrane hyperpolarizes as long as the peptide is applied.

p0445 If now a negative current is applied through the recording pipette which contains a KCl solution, Cl^- ions are expelled from the pipette. They hyperpolarize the membrane (**Figure 3.11**). The electrical circuit is closed because ions can go through the membrane via the channels open at rest.

st0135

3.8 SUMMARY

p0450 Passage of ions through the membrane is a regulated process and the flow of ions across the neuronal plasma membrane is not a simple and anarchic diffusion through a lipid bilayer. Instead, it is restricted through transmembrane proteins whose opening (channel proteins) or activation (pumps or transporters) are tightly controlled by different factors.

st0140 ***Where and how do ions passively cross the plasma membrane? (See also Appendices 3.2 and 3.3)***

p0455 • Ions move passively across the plasma membrane through ionic channels that are specifically permeable to one or several ions of the same sign. They move down their electrochemical gradient. This passive movement of charges is a current that can be recorded. Through a single channel it is a unitary current i_{ion}, and through N channels it is a macroscopic current or total current I_{ion}.

u0155 • The type of ion that moves through an open channel (ionic selectivity of the channel pore) is determined by the structure of the channel itself. This ionic selectivity gives the name to the channel. For example, an Na^+ channel is permeable to Na^+ ions; a cationic channel is permeable to cations: Na^+, K^+ and sometimes also Ca^{2+}.

u0160 • The *direction* of ion diffusion through a single channel depends on the electrochemical gradient or driving force for this particular ion ($V_m - E_{ion}$).

u0165 • The *number* of charges that diffuse through an open channel per unit of time (i_{ion}) depends on the electrochemical gradient ($V_m - E_{ion}$) but also on how easily ions move through the pore of the channel (expressed as the conductance γ_{ion} of the channel): $i_{ion} = \gamma_{ion}(V_m - E_{ion})$.

st0145 ***How and where do ions actively cross the plasma membrane and thus compensate for the passive movements? (see also Appendix 3.1)***

p0480 Active movements of Na^+, K^+, Ca^{2+} or Cl^- ions across the membrane occur through pumps or transporters. Pumps obtain energy from the hydrolysis of ATP, whereas transporters use the energy of an ionic gradient, for example the sodium driving force. These transports require energy since they operate against the electrochemical gradient of the transported ions or molecules. They maintain ionic concentrations at constant values in the extracellular and intracellular compartments despite the continuous passive movements of ions across the membrane.

st0150 ***What are the roles of electrochemical gradients and passive movements of ions?***

p0485 The electrochemical gradients of ions are a reserve of energy: they allow the existence of ionic currents and drive some active transports. The large asymmetries in ion distribution imply a dynamic state through which cell-to-cell signaling is made possible. Ionic currents have two main functions: (i) they evoke transient changes of membrane potential which are electrical signals of the neuron (action potentials or postsynaptic potentials or sensory potentials) essential to neuronal communication; and (ii) they locally increase the concentration of a particular ion in the intracellular medium, for example Ca^{2+} ions, and thus trigger intracellular Ca^{2+}-dependent events such as secretion or contraction.

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APPENDIX 3.1 THE ACTIVE TRANSPORT OF IONS BY PUMPS AND TRANSPORTERS MAINTAIN THE UNEQUAL DISTRIBUTION OF IONS

p0490 Passive movements of Na^+, K^+, Ca^{2+} or Cl^- ions across the membrane would finally cause concentration changes in the extracellular and intracellular compartments if they were not constantly regulated during the entire life of the neuron by transport of ions in the reverse direction, against passive diffusion; i.e. against electrochemical gradients. This type of transport is described as active since it requires energy in order to oppose the electrochemical gradient of the transported ions. Ions cross the membrane *actively* through specialized proteins known as pumps or transporters. Pumps obtain energy from the hydrolysis of ATP, whereas transporters use the energy of an ionic gradient, for example the sodium driving force (**Figure A3.1**). The energy is needed for the conformational changes that allow the pump or the transporter to change its affinity for the ion transported during the transport: the binding site(s) must have a high affinity when facing the medium where the transported ion is at a low concentration (in order to bind it) and must change to low affinity when facing the medium where the concentration of the transported ion is high in order to release it.

A3.1.1 Pumps are ATPases that actively transport ions

st0160

p0495 Pumps have ATPase activity (they hydrolyze ATP). This ATPase activity is generally the easiest way of identifying them. Pumps are membrane-embedded enzymes that couple the hydrolysis of ATP to active translocation of ions across the membrane. The central issue of ion motive ATPases is to couple the hydrolysis of ATP (and their auto-phosphorylation) to the translocation of ions.

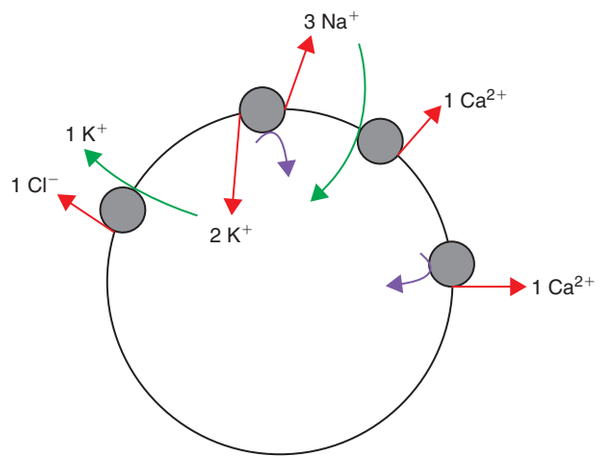

FIGURE A3.1

f0065 Transport against the electrochemical gradient of the ion

o0010 Transport along the electrochemical gradient of the ion

o0015 ATP hydrolysis

The Na/K-ATPase pump

st0165

p0500 Na/K-ATPases maintain the unequal distribution of Na^+ and K^+ ions across the membrane. Na^+ and K^+ ions cross the membrane through different Na^+ and K^+ permeable channels (voltage-sensitive Na^+ and K^+ channels plus receptor channels). This pump operates continuously at a rhythm of 100 ions per second (compared with 10^6–10^8 ions per second for a channel), adjusting its activity to the electrical activity of the neuron. It actively transports three Na^+ ions towards the extracellular space for each two K^+ ions that it carries into the cell.

p0505 The energy of ATP hydrolysis is needed for the conformational changes (they are energy dependent) that allow the pump to change its affinity for the ion transported, whether the binding sites are accessible from the cytoplasmic or the extracellular sides. For example, when the Na^+ binding sites are accessible from the cytoplasm, the protein is in a conformation with a high affinity (K_A = 1 mM) for intracellular Na^+ ions, and so Na^+ ions bind to the three sites. In contrast, when the three Na^+ have been translocated to the extracellular side, the protein is in a conformation with a low affinity for Na^+ ions so that the three Na^+ are released in the extracellular space.

p0510 The steady unequal distribution of Na^+ and K^+ ions constitutes a reserve of energy for a cell. The neuron uses this energy to produce electric signals (action potentials, synaptic potentials) as well as to actively transport other molecules.

The Ca-ATPase pump

st0170

p0515 The function of Ca-ATPases is to maintain (with the Na–Ca transporter) the intracellular Ca^{2+} concentration at very low levels by active expulsion of Ca^{2+}. In fact, the intracellular Ca^{2+} concentration is 10 000 times lower than the extracellular concentration despite the inflow of Ca^{2+} (through receptor channels and voltage-gated Ca^{2+} channels) and the intracellular release of Ca^{2+} from intracellular stores. Maintaining a low intracellular Ca^{2+} concentration is critical since Ca^{2+} ions control several intracellular reactions and are toxic at a high concentration. Ca-ATPases are located in the plasma membrane and in the membrane of the reticulum. The former extrude Ca^{2+} from the cytoplasm whereas the latter sequester Ca^{2+} inside the reticulum (see also **Figure 7.8**).

A3.1.2 Transporters use the energy stored in the transmembrane electrochemical gradient of Na^+, K^+, H^+ or other ions

st0175

p0520 When transporters carry Na^+, K^+ or H^+ ions (along their electrochemical gradient) in the same direction as

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the transported ion or molecule, the process is called *symport*. When the movements occur in opposite directions, the process is called *antiport*. We shall study only transporters implicated in the electrical or secretory activity of neurons.

st0180 ***The Na–Ca transporter***

p0525 This transporter uses the energy of the Na^+ gradient to actively carry Ca^{2+} ions towards the extracellular environment. It is situated in the neuronal plasma membrane and operates in synergy with the Ca-ATPase and with transport mechanisms of the smooth sarcoplasmic reticulum to maintain the intracellular Ca^{2+} concentration at a very low level (see Section 7.2.4).

st0185 ***The K–Cl transporter KCC***

p0530 Adult mammalian central neurons maintain a low intracellular Cl^- concentration. Cl^- extrusion is achieved by K^+–Cl^- cotransporters (KCC) fuelled by K^+. As all transporters, it does not directly consume ATP but derives its energy from ionic gradients, here the K^+ gradient generated by the Na/K/ATPase.

st0190 ***Neurotransmitter transporters***

p0535 Inactivation of most neurotransmitters present in the synaptic cleft is achieved by rapid reuptake into the presynaptic neural element and astrocytic glial cells. This is performed by specific neurotransmitter transporters, transmembrane proteins that couple neurotransmitter transport to the movement of ions down their concentration gradient. Certain neurotransmitter precursors are also taken up by this type of active transport (glutamine and choline, for instance). Once in the cytoplasm, neurotransmitters are concentrated inside synaptic vesicles by distinct transport systems driven by the H^+ concentration gradient (maintained by the vesicular H^+-ATPase) (see Section 7.4).

st0195

APPENDIX 3.2 THE PASSIVE DIFFUSION OF IONS THROUGH AN OPEN CHANNEL

p0540 It has been stated above that a channel is said to be in a closed state (C) when its ionic pore does not allow ions to pass. In contrast, when the channel is said to be in the open state (O), ions can diffuse through the ionic pore.

$$C \rightleftharpoons O$$

p0545 This diffusion of ions through an open channel is a passive transport since it does not require energy expenditure.

u0170 • Which type(s) of ions will move through a given open channel: cations, anions?

u0175 • In which direction will these ions move, from the external medium to the cytosol or the reverse?

u0180 • How many of these ions will move per unit of time?

st0200 ***The structure of the channel pore determines the type of ion(s) that diffuse passively through the channel***

p0565 The pores of ion channels select their permeant ions. The structural basis for ion channel selectivity has been studied in a bacterial K^+ channel called the KcsA channel (it is a voltage-independent K^+ channel). All K^+ channels show a selectivity sequence $K^+ = Rb^+ > Cs^+$, whereas permeability for the smallest alkali metal ions Na^+ and Li^+ is extremely low. Potassium is at least 10 000 times more permeant than Na^+, a feature that is essential to the function of K^+ channels. Each subunit of the KcsA channel consists of an N-terminal cytoplasmic domain, followed by two transmembrane helices and a C-terminal globular domain in the cytoplasm. The P loop (P for pore) situated between transmembrane helices 1 and 2 is the region primarily responsible for ion selectivity.

p0570 The KcsA channel is overexpressed in bacteria and the three-dimensional structure of its pore investigated by the use of X-ray crystallography. The KcsA channel is a tetramer with fourfold symmetry around a central pore (**Figure A3.2**). The pore is constructed of an inverted teepee with the extracellular side corresponding to the base of the teepee. The overall length of the pore is 4.5 nm and its diameter varies along its distance. From inside the cell the pore begins as a water-filled tunnel of 1.8 nm length (inner pore) surrounded by predominantly nonpolar side chains pointing to the pore axis. The diameter of this region is sufficiently wide to allow the passage of fully hydrated cations. This long entry way then opens

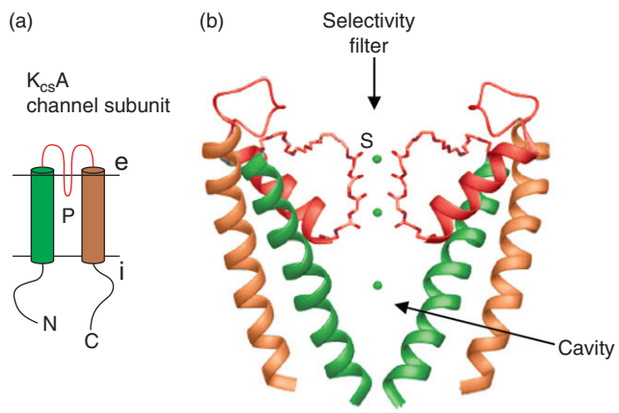

FIGURE A3.2 (**a**) Membrane topology of the KcsA channel subunit showing the two transmembrane segments and the pore loop (P). (**b**) Two diametrically opposed subunits of KcsA are depicted to show the cavity in the membrane with 3 ions in the cavity, S sites (ion-binding sites) of the selectivity filter (shown in sticks). *Adapted from Noskov SY, Roux B (2006) Ion selectivity in potassium channels. Biophys. Chem.* **124**, *279-291, with permission.* f0070

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to a wider water-filled cavity (1 nm across). Beyond this vestibule is the 1.2 nm long selectivity filter. After this, the pore opens widely to the extracellular side of the membrane.

st0205 ***What are the respective roles of the parts of the pore?***

p0575 The pore comprises a wide, non-polar aqueous cavity on the intracellular side, leading up, on the extracellular side, to a narrow pore that is 1.2 nm long and lined exclusively by main chain carbonyl oxygens formed by the residues corresponding to the signature sequence TTV-GYG common to all K^+ channels.

p0580 Electrostatic calculations show that when an ion is moved along a narrow pore through a membrane it must cross an energy barrier that is maximal at the membrane center. A K^+ ion can move throughout the inner pore and cavity and still remain mostly hydrated, owing to the large diameter of these regions. The role of the inner pore and the cavity is to lower the electrostatic barrier. The cavity overcomes the electrostatic destabilization from the low dielectric bilayer by simply surrounding an ion with polarizable water. Another feature that contributes to the stabilization of the cation at the bilayer center are the four pore helices which point directly at the center of the cavity. The amino to carboxyl orientation of these helices imposes a negative electrostatic (cation attractive) potential via the helix dipole effect. These two mechanisms (large aqueous cavity and oriented helices) serve to stabilize a cation in the hydrophobic membrane interior.

p0585 The selectivity filter that follows, in contrast, is lined exclusively by polar main-chain atoms. They create a stack of sequential carbonyl oxygen rings which provide multiple closely spaced binding sites (S) for cations separated by 0.3–0.4 nm. This selectivity filter attracts K^+ ions and allows them to move.

st0210 ***Why are cations permeant and not anions?***

p0590 As might have been anticipated for a cation channel, both the intracellular and extracellular entryways are negatively charged by acidic amino acids that raise the local concentration of cations while lowering the concentration of anions.

st0215 ***Why are K^+ ions at least 10 00 times more permeant than Na^+ ions?***

p0595 The selectivity filter is so narrow that a K^+ ion evidently dehydrates to enter into it and only a single K^+ ion can pass through at one time. To compensate for the energy cost of dehydration, the carbonyl oxygen atoms come in very close contact with the ion and act like surrogate water – they substitute for the hydration waters of K^+. This filter is too large to accommodate an Na^+ ion with its smaller radius (main chain oxygens are spatially inflexible and their relative distances to the center of the pore cannot readily be changed). It is proposed that a K^+ ion fits in the filter so precisely that the energetic costs and gains are well balanced.

st0220 ***What drives K^+ ions to move on?***

p0600 K^+ ions bind simultaneously at two binding sites 0.75 nm apart near the entry and exit point of the selectivity filter. Binding at adjacent sites may provide the repulsive force for ion flow through the selectivity filter: two K^+ ions at close proximity in the selectivity filter repel each other. The repulsion overcomes the strong interaction between ion and protein and allows rapid conduction in the setting of high selectivity. This leads to a rate of diffusion of around 10^8 ions per second.

APPENDIX 3.3 THE NERNST EQUATION st0225

p0605 The material in this appendix is adapted from Katz B (ed.) (1966) *Nerve, Muscle and Synapse* (New York: McGraw-Hill). When $V_m = E_{ion}$, a particular ion has an equal tendency to diffuse in one direction according to its concentration gradient as to move in the reverse direction according to membrane potential. The net flux of this ion is null, so the current carried by this ion is null. $V_m = E_{ion}$ means that:

$$\text{osmotic work}(W_o) = \text{electrical work}(W_e) \qquad \text{(a)}$$

p0610 The osmotic work required to move one mole of a particular ion from a compartment where its concentration is low to a compartment when its concentration is high is equal to the electrical work needed to move one mole of this ion against the membrane potential in the opposite direction. Here, active diffusion of ions is considered instead of passive diffusion. The electrical work required to move 1 mole of an ion against a potential difference E_{ion} is:

$$W_e = zFE_{ion}, \qquad \text{(b)}$$

where z is the valence of the transported ion, equal to +1 for monovalent cations such as Na^+ or K^+, to −1 for monovalent anions such as Cl^{-}, and to +2 for divalent cations such as Ca^{2+}. F is the Faraday constant. F for hydrogen is the charge of one hydrogen atom: $F = Ne$. Here N is the Avogadro number, which is 6.022×10^{23} mol^{-1} (one mole of hydrogen atoms contains 6×10^{23} protons and the same number of electrons), and e is the elementary charge of a proton, which is 1.602×10^{-19} coulombs (C). So $F = 96\,500$C mol^{-1}. Therefore zF with $z = 1$ is the charge of 1 mole of protons or 1 mole of monovalent cations (Na^+, K^+). The charge of one mole of monovalent anions (Cl^-) is $-F$ ($z = -1$); the charge of 1 mole of divalent cations (Ca^{2+}) is $2F$ ($z = 2$); etc.

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p0615 The osmotic work required to move 1 mole of ions from a compartment where its concentration is low to a compartment where the concentration is high can be compared to the work done in compressing 1 g equivalent of an ideal gas. The gas is contained in a cylinder with a movable piston. Mechanical work to move the piston is W, calculated from force times distance of displacement of the piston (δl). The force exerted is equal to the pressure p of the gas multiplied by the surface area S of the piston. So the work δW done to displace the piston is $pS\ \delta l$, which equals $p\ \delta v$. Therefore the work done in compressing a gas from a volume v_1 to a volume v_2 is:

$$W = \int_{v2}^{v1} p\, dv. \qquad \text{(c)}$$

p0620 The gas law tells us that $pv = RT$ (hence $p = RT/v$), with R the constant of an ideal gas (R = 8.314 V C K^{-1} mol^{-1}) and T is the absolute temperature.

p0625 Equation (c) can be changed to:

$$W = RT \int_{v2}^{v1} (1/v)\, dv = RT(\ln v_1 - \ln v_2) \qquad \text{(d)}$$
$$= RT \ln(v_1/v_2).$$

p0630 By analogy the osmotic work is:

$$W_o = RT \ln([\text{ion}]_e / [\text{ion}]_i) \qquad \text{(e)}$$

p0635 From equation (a), $W_o = -W_e$, so from equations (b) and (e) the Nernst equation is obtained:

$$RT \ln([\text{ion}]_e / [\text{ion}]_i) = zFE_{\text{ion}}$$

$$E_{\text{ion}} = (RT / zF) \ln([\text{ion}]_e / [\text{ion}]_i) \qquad \text{(Nernst)}$$

p0640 At 20°C, RT/F is about 25 mV, and moving from Neperian logarithms to decimal ones a factor of 2.3 is needed. Hence:

$$E_{\text{ion}} = (58 / z) \log_{10}([\text{ion}]e / [\text{ion}]_i).$$

p0645 Of course, this description of the E_{ion} is entirely based on a physical theory of passive ion movements. Transmembrane flux of ions, however, involves active transport of ions as well. For example, the gradients for Na^+ and, in particular, for Ca^{2+} are regulated by complex mechanisms relying on transporters and intracellular sequestration so that the possibility of predicting the precise reversal potential of responses mediated by rises in Na^+ or Ca^{2+} permeability on the basis of their apparent transmembrane concentrations is limited.

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Non-Print Items

Abstract

The neuronal plasma membrane delimits the whole neuron, cell body, dendrites, dendritic spines, axon and axon terminals. It is a barrier between the intracellular and extracellular environments. The general structure of the neuronal plasma membrane is similar to that of other plasma membranes. It is made up of proteins inserted in a lipid bilayer, forming as a whole a 'fluid mosaic'. However, insofar as there are functions that are exclusively neuronal, the neuronal membrane differs from other plasma membranes by the nature, density and spatial distribution of the proteins of which it is composed. The presence of a large diversity of transmembrane proteins called *ionic channels* (or simply 'channels') characterizes the neuronal plasma membrane. They allow the passive movement of ions across membranes and thus electrical signaling in the nervous system.

Keywords: active transport; anion; cation; conductance; depolarization; hyperpolarization; ionic channel; membrane potential; membrane protein; neuronal plasma membrane; passive transport; repolarization; resistance; resting membrane potential; reversal potential of an ion; Nernst equation; transporter

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