Theory of action potentials

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ii

Contents

2	The	e membrane potential	1
	2.1	Elements of neuronal cell biology	1
		2.1.1 Morphology	1
		2.1.2 Structure of a neuron \ldots	5
	2.2	Membrane polarization	1
		2.2.1 A membrane permeable to K^+	2
		2.2.2 A membrane permeable to K^+ and Cl^-	6
		2.2.3 A membrane permeable to K^+ , Cl^- and Na^+	6
		2.2.4 Ion pumps	0
	2.3	Osmosis and mechanics	1
	2.4	Summary and epistemological notes	3

CONTENTS

iv

Chapter 2

The membrane potential

2.1 Elements of neuronal cell biology

In this book, we will focus mainly on neurons. However, the matter covered in this chapter also applies to a large extent to non-neuronal cells, such as muscle fibers and excitable unicellular organisms.

2.1.1 Morphology

All living organisms are made of cells bound by membranes. Cells come in various sizes and shapes¹. The bacterium *E. Coli* is roughly cylindrical with a diameter of 1 μ m and a length of 2 μ m. In humans, small lymphocytes are approximately 7 μ m in diameter while large liver cells reach 50 - 100 μ m. Most animal cells are 10 - 30 μ m in diameter. As we have seen in section ??, many non-neural cells are excitable and can produce APs (for example muscle fibers), but in this book we will focus on neurons. Neurons are excitable cells that have extensions called neurites (axons and dendrites), which can extend over very long distances. For example, axons of the human sciatic nerve extend from the base of the spinal cord to the big toe of each foot, which makes them around 1 m long.

Figure 2.1 shows a particular type of neuron from the human cerebellum called a Purkinje cell, drawn at the end of the 19th century by Ramon-y Cajal (1899) using Golgi staining. Other drawings from the same book are shown in figures 2.2, 2.3 and 2.5 (note that neurons in Fig. 2.3 are not to scale). A neuron is composed of a cell body or *soma*, which includes the nucleus where DNA is located², and extensions called *neurites*. There are two types of neurites: axons and dendrites. The Purkinje cell has a soma of about 25–40 μ m (in rats (Herndon, 1963)) and, as most vertebrate neurons, it has a single axon stemming from the soma³, which then branches and reaches other neurons. APs are produced at the beginning of the axon, in a structure called the *axon initial segment* (AIS), and then propagates along the axon towards other neurons. The diameter of this axon is about 1.5 μ m. Other axons in the nervous system vary in diameter in the 0.1 – 10 μ m range⁴ (Perge et al., 2012). The lower limit of 0.1 μ m might be determined by biophysical constraints, in particular the stochasticity of ion channel opening (Faisal et al., 2005)

¹Morphological reconstructions of neurons can be found on http://neuromorpho.org/.

²Procaryotes (bacteria) do not have a nucleus; eucaryotes, which include all multicellular organisms, do.

 $^{^{3}}$ Axons also frequently originate from a dendrite (Husser et al., 1995; Gentet and Williams, 2007; Thome et al., 2014).

 $^{^{4}}$ with special cases such as the squid giant axon, with a diameter of about 1 mm.



Figure 2.1: Purkinje cell of the human cerebellum.



Figure 2.2: Pyramidal cortical neurons. A, Neuron from a rabbit. B, Adult neurons from different species (A to D: frog, sand lizard, rat, man) and different development stages.

(see chapter ??). The Purkinje cell also has a highly branched structure called the *dendritic tree*. The axon of a neuron contacts the membrane of other neurons at *synapses*, which are generally located on dendrites and on the soma⁵. A single Purkinje cell has on the order of 100,000 synapses. Current is generated at the synapse on the postsynaptic side (target neuron) when an AP arrives on the presynaptic side (source neuron). Thus, the general direction of electrical flow goes from the dendrites to the soma, then through axon to the axonal terminals (where synapses are made onto target neurons). As already mentioned in chapter ??, the AP also often backpropagates from the axonal site of initiation into the soma and dendrites (Stuart and Sakmann, 1994). The dendritic tree of the Purkinje cell extends over about 200 μ m, but this is highly variable between cell types. Dendrite diameter is a few μ m at the base (near the soma), and decreases below the μ m at the tips.

This general structure is found in most vertebrate neurons, with various dimensions and shapes. Figure 2.3A shows the principal excitatory neuron of the cortex, called a *pyramidal cell*. The cell body is about 20 μ m in diameter. It extends a long dendritic tree towards the surface of the cortex, covered by *spines*, which are small protusions (< 1 μ m) typically receiving a single synapse. There are about 10,000 synapses on a pyramidal cell. The axon is the spineless neurite originating from the soma⁶. Figure 2.3B shows pyramidal cells from different species (A-D) and different stages of development (a-e). As can be seen, the general structure is similar between species, with differences in the branching structure of both dendrites and axons.

Figure 2.3 shows these two cell types in the context of the motor circuit in which they are

⁵There are also synapses on the axon, in particular on the AIS (Kosaka, 1980).

 $^{^{6}}$ and sometimes from a basal dendrite (Thome et al., 2014).



Figure 2.3: Neurons of the motor pathway (top to bottom: brain, cerebellum, spine and muscles). Pyramidal cortical cells are shown on top (A) and Purkinje cells on the right (C).



Figure 2.4: Neurons of the retina: a; cones; b, rods; c and d: bipolar cells; e: ganglion cells; f: amacrine cells.

embedded. Arrows show the direction of AP propagation. Pyramidal cells project long axons to the spine, where they contact the dendrites of motoneurons, which in turn project axons to muscles. The axons of pyramidal cells also branch in the brain towards the cerebellum, where they indirectly contact Purkinje cells. Purkinje cells also project axons towards motoneurons of the spine. Dendrites are always somewhat local (extending over less than 1 mm), while axons can extend over distances of that are comparable with the organism's size, up to about 1 meter in human motoneurons.

Many neurons also have a somewhat different anatomical structure, as exemplified by neurons of the retina (Figure 2.4). For example, amacrine cells (f) have no axon, only a dendritic tree extending over $70 - 350 \ \mu\text{m}$. Ganglion cells (e) are the only retinal cells whose axon projects outside the retina. Bipolar cells (c) have a single long dendrite originating from the soma and then branching near the photoreceptors (a and b), and an axon on the opposite side. Neurons that constitute the auditory nerve, the spiral ganglion cells, are also bipolar, but both neurites are excitable; APs initiate near the synapse with the inner hair cells that sit on the cochlea (Hossain et al., 2005), then propagate to the soma, then continue propagating towards axonal terminals in the brainstem.

In invertebrates, the cell body is not on the path of electrical propagation between synapses and axonal terminals (Figure 2.5). Instead, neurons are generally *unipolar*, with a single neurite emerging from the soma, which serves both as axon and dendrite carrying synapses (there are also dendritic branches).

2.1.2 Structure of a neuron

The membrane

The membrane of an animal cell is on the order of 5 nm thick and is made of two layers of lipid molecules. Those molecules have a hydrophilic end and a hydrophobic end, and so they



Figure 2.5: Earthworm motoneuron contacted by a sensory neuron (arrows show the direction of APs).



Figure 2.6: The fluid mosaic model of membranes, showing proteins embedded into a lipid bilayer (Singer and Nicolson, 1972).

2.1. ELEMENTS OF NEURONAL CELL BIOLOGY

spontaneously arrange themselves by pair, with their hydrophobic ends against each other. About half of the mass of cell membranes is made of proteins, which are embedded into the lipid bilayer (e.g. ion channels) or attached to the membrane. Current understanding of the structure of cell membranes is described by the *fluid mosaic model* (Figure 2.6) (Singer and Nicolson, 1972): the membrane is seen as a two-dimensional solution of proteins, where lipids constitute the solvent. Thus, both lipids and proteins can move by diffusion (random walk) in the membrane.

However, there are restrictions on membrane diffusion, first because the membrane is crowded, and second because some constituents of the membrane are attached to the cytoskeleton, the scaffolding of the cell (Jacobson et al., 1995). For example, in the axon initial segment, superresolution microscopy has revealed that sodium channels are anchored to a scaffold protein called ankyrin-G, which is attached to a periodic structure made of actin rings separated by pairs of β -IV spectrin molecules (Xu et al., 2013; Leterrier et al., 2015) (Figure 2.7). The dense cytoskeleton of the AIS also forms a barrier to membrane diffusion (Nakada et al., 2003), which prevents proteins from moving freely from the soma to the axon membrane.

The membrane separates the inside of the cell, called the *cytoplasm*, from the extracellular medium. Water and some uncharged molecules (e.g. urea, gases) can move across the membrane, but a critical property of cell membranes is that proteins cannot freely move across them: this ensures that the biological material of the cell does not diffuse into the extracellular medium. Other molecules and in particular small ions such as Na⁺ and K⁺ are also blocked by the bilipidic layer, but K⁺ ions can cross the membrane through channels, which are transmembrane proteins (more detail in section 2.2). This makes the membrane permeable to K⁺, while the permeability to other ions is low at rest.

Inside neurons

The content of a cell is called the *cytoplasm*. It encloses a number of organelles bathing in a liquid substance called the *cytosol* (Figure 2.8). In eukaryotes (including all multicellular organisms), one of these organelles is the nucleus, located in the soma, which includes the genetic material and is the site of transcription, the first step in gene expression where the DNA of a gene is copied into messenger RNA (mRNA). The fact that transcription is localized to the nucleus is significant for neurons, because in order to produce new membrane channels in distal regions of the axon, mRNA must be transported over very long distances. As we already noted in section **??**, the speed of axonal transport does not exceed a couple of μ m/s or about 200 mm per day. Therefore, regulation of genetic transcription has a slow effect on the electrical properties of axons. This will become important when we discuss the development and maintenance of excitability in chapter **??**.

Proteins such as ion channels are synthesized from mRNA in *ribosomes*, which are organelles present in all the cytoplasm including the axon. *Mitochondria* produce the energy currency of the cell, ATP, through respiration. There are a number of other types of organelles, which are less directly relevant for the subject of this book.

The cytoplasm also includes the *cytoskeleton* of the cell, which is the scaffolding that gives the cell its shape and mechanical properties. Without the cytoskeleton, there would be no neurite. The cytoskeleton includes *microtubules* (filaments in Figure 2.8), which form a sort of railway network used for the transport of biological material, in addition to providing mechanical support.

The cytosol is the liquid content of the cell, and represents about 70% of the cell's volume. Bernstein's membrane theory (section ??) sees the cytosol as a watery solution of small ions (mainly K^+). Although the cytosol is indeed mostly water, it is crowded with proteins (about 20% in volume), including the cytoskeleton, which has some effect on the properties of the dis-



Figure 2.7: Axon cytoskeleton. A, Periodic structure of the axon cytoskeleton, with actin rings (green) connected by β -IV spectrin (purple) (Xu et al., 2013). B, Molecular structure of the axon initial segment, with sodium (Nav) channels embedded into the membrane and anchored to ankyrin-G (Leterrier et al., 2015).



Figure 2.8: Soma of a Purkinje cell, showing the nucleus and various organelles (Herndon, 1963).

CHAPTER 2. THE MEMBRANE POTENTIAL

		Intracellular concentration (mM)	Extracellular concentration (mM)	E_S (mV)
Squid axon	K^+	410	10	-93
	Na^+	49	460	56
	Cl^-	40	540	-65
Cat motoneuron	\mathbf{K}^+	150	5.5	-83
	Na^+	15	150	58
	Cl^-	9	125	-66

Table 2.1: Concentrations of the main ions of the cytosol and extracellular medium in the squid giant axon (Hodgkin, 1951) and the cat spinal motoneuron (Eccles, 1957), and corresponding Nernst potentials E_S at $T = 20^{\circ}$ C (see section 2.2.1).

solved molecules⁷. For example, ions diffuse more slowly in the cytosol than in water (Verkman, 2002). This situation is called *macromolecular crowding*. We will not explore these effects further and in the following we will mostly consider the cytosol as a solution, but it is important to keep in mind that it is not an ideal solution.

The free water of the cytosol contains many ions, which are molecules that carry an electrical charge. For example, when salt is dissolved in water, the NaCl molecule (sodium chloride) splits into two ions, Na⁺ and Cl⁻. Positively charged ions are called *cations*, negatively charged ions are called *anions*. Cells are approximately electrically neutral at all times (see section 2.2), which means that the total charge carried by anions matches the total charge carried by cations. The ionic composition of the cytosol differs between cells and is generally not known with great precision. Table 2.1 gives the approximate concentrations of K⁺, Na⁺ and Cl⁻ in two cells: the squid giant axon (Hodgkin, 1951) and the cat spinal motoneuron (Eccles, 1957). Other mammalian cells have similar ionic composition to the spinal motoneuron. As proposed by Bernstein, the main intracellular cation in all cells is K⁺ with a concentration of about 100 - 150 mM⁸ in mammalian cells.

We note that the ionic composition of the cytosol given in table 2.1 is not electrically neutral. In reality, the cytosol is electrically neutral: the positive charges (mainly K^+) are balanced by an equal number of negative charges, in the form of large ions (phosphate HPO_4^{2-} , bicarbonate HCO_3^{-} , sulfate SO_4^{2-}), charged proteins and amino acids. Part of these charges are essentially immobilized, and therefore do not participate in electrical currents.

Outside neurons

Extracellular space also contains ions dissolved in water (table 2.1), mainly Na⁺ and Cl⁻. It is sometimes speculated that the ionic composition of extracellular space in vertebrates reflects the composition of ancient oceans. Thus there are large gradients of ionic concentration across the membrane: high concentration of Na⁺ outside the cell vs. high concentration of K⁺ inside the cell. The extracellular space also contains a collection of molecules such as collagen, which provide structural support to the cells (among other functions), collectively called the extracellular matrix (ECM).

In addition to neurons, the nervous system also includes *glial cells*, collectively called *glia*, which are more numerous than neurons. Glial cells are not excitable but have many functions,

 $^{^{7}}$ Hodgkin described the cytosol of the squid giant axon as a gel (Hodgkin, 1964), but the modern conception is more that of a highly crowded solution.

⁸Millimolar (mM) is the commonly used unit for molar concentration in biology: 1 molar is 1 mol/L; 1 mM is 1 mol/m³. One mole (mol) is about 6.0221404×10^{23} molecules (the Avogadro number).



Figure 2.9: A simple chemical model of a cell (A⁻ represents organic anions).

including: providing nutrients and oxygen to neurons, regulating ion homeostasis in extracellular space, participating in synaptic plasticity, insulating axons. The latter is most directly relevant to the subject of this book. A particular type of glial cells called *oligodendrocytes* (Schwann cells in the peripheral nervous system) coat the axons of some neurons with *myelin*, an insulating layer which belongs to the membrane of oligodendrocytes. We will cover this subject in more detail when we discuss the propagation of APs in chapter ??.

2.2 Membrane polarization

In a simplified view, the cell can thus be seen as a membrane separating two electrolytes, the internal medium or cytosol containing mostly K^+ and the external medium containing mostly Na^+ and Cl^- (Figure 2.9). At rest, the membrane is permeable mostly to K^+ ions, and to a lesser extent to Cl^- and Na^+ . These ions can pass through *ionic channels*. In this chapter, we only consider membrane properties that do not involve voltage-dependent movements of ionic channels, which we will study in chapter ??. These are called *passive properties* of the membrane because membrane permeabilities are considered constant. We generally use the terms *active properties* to refer to voltage-dependent permeability changes, such as those underlying the action potential. This nomenclature can be slightly misleading because the channel movements that underlie "active" properties do not require the cell to provide energy (the energy is provided by the electrical field). In contrast, we will see in section 2.2.4 that the maintenance of concentration gradients across the membrane, and therefore membrane polarization, requires "active" pumps, which consume energy in the form of ATP. Let us simply note that when referring to the membrane, the terms passive vs. active mean constant vs. voltage-dependent permeabilities.

As outlined in section ??, the permeability of the membrane to K^+ combined to the gradient of K^+ concentration across the membrane results in the establishment of a membrane potential, i.e., of an electrical field across the membrane. We will now explain the biophysics of this phenomenon in detail.



Figure 2.10: Ionic diffusion through a membrane channel. The shading corresponds to the concentration of K^+ (higher on the intracellular side).

2.2.1 A membrane permeable to K^+

In general, it is not trivial to calculate the membrane potential that develops through a semipermeable membrane, because it depends on the detailed properties of the membrane, in particular of the ionic channels. However, there is one simple case when this calculation is relatively easy: the case of a membrane that is permeable to a single ion species. In this case, the membrane potential depends only on the ion concentrations on both sides of the membrane, and not so much on the specificities of the channels. The value of this membrane potential is called the *Nernst potential* or *reversal potential* for that ion species. The reason why it is called reversal potential will become clearer when we explain the phenomenon.

The Nernst potential can be derived in several ways from physical considerations. These derivations can be found in many textbooks and are not particularly important in themselves to understand action potentials. However, we will study one of these derivations in detail because it will be the opportunity to illustrate the two mechanisms at play, diffusion and electrical forces. Here we will focus on the case when the membrane is permeable to K^+ , but the analysis also applies to a membrane permeable to another single ionic species.

Diffusion

Let us start with a membrane that separates two ionic solutions, representing the cytosol and the extracellular space. We assume that the membrane is only permeable to K^+ and that the two solutions contain different concentrations of K^+ , which we denote $[K^+]_i$ (inside) and $[K^+]_o$ (outside). These ions move randomly by thermal agitation or *diffusion*. Macroscopically, this random motion has the consequence that molecules flow from regions of high concentration to regions of low concentration. To see this, imagine there is a cylindrical channel across the

Diffusion coefficient $(10^{-5} \text{ cm}^2/\text{s})$

Na^+	1.33
K^+	1.96
Cl^-	2.03
Ca^{2+}	0.79

Table 2.2: Diffusion coefficients measured in water at 25°C (Hille, 2001).

membrane, of length L, through which K^+ ions can pass (Figure 2.10). The concentration of ions along the tube is denoted C(x), where $C(0) = [K^+]_i$ and $C(L) = [K^+]_o^9$. We assume that ions move randomly and independently through the tube by thermal agitation. How many ions move through position x in the positive direction in a given time interval dt? This number is actually the difference between the ions that move through x in the positive direction, and the ions that move through x in the negative direction. Therefore, it must be proportional to the difference in the number of ions just before x and just after x, over a small length dx, and therefore it is proportional to C(x) - C(x + dx). It is also clearly proportional to the section area of the channel. Thus, the flux of ions in the positive direction at position x, per unit time and section area is:

$$J_D(x) = -D\frac{\partial C}{\partial x}$$
 (Fick's first law)

where the proportionality coefficient D is called the *diffusion coefficient*. This formula is called *Fick's first law*, and it generalizes to two and three dimensions by replacing the spatial derivative by a gradient. The diffusion coefficient is in units of m².s⁻¹, since the flux J is units of mol.s⁻¹.m⁻² and concentration C is in units of mol.m⁻³.

What does this coefficient mean physically at a molecular level? It quantifies how far an ion move on average in a given amount of time. Ions move by Brownian motion or random walk, which means that the total movement an ion performs in a time interval t is a sum of small independent random movements. This implies that the variance $\langle x^2 \rangle$ of its position grows linearly with t. The proportionality coefficient is 2D, that is, $\langle x^2 \rangle = 2Dt$. Therefore, $D = \langle x^2 \rangle /(2t)$. This diffusion coefficient depends on the ionic species (in particular, heavier molecules have a lower diffusion coefficient), it increases linearly with temperature, and it also depends on the solvent. A few diffusion coefficients measured in water at 25°C are listed in table 2.2. Remember that these are likely to be lower, in the crowded cytosol. For example, in frog muscle fibers, diffusion coefficients of Na⁺ and K⁺ are about twice smaller (Kushmerick and Podolsky, 1969).

Diffusion tends to equalize the ionic concentrations on the two sides of the membrane. However, since ions carry electric charges, their displacement produces an electrical field, which then exerts a force on the ions.

The electrical field

Each charge produces an electrical field, which exerts a force on all other charges. Charges of the same sign repel each other, while charges of different signs attract each other. In an electrically neutral environment, the forces produced by positive and negative charges cancel each other. However, when K^+ ions leave the membrane by diffusion, they create an excess of positive charges in the immediate vicinity of the membrane, which then produces an electrical field **E** (we represent vectors with bold face).

⁹To be more realistic, one would assume that $C(0) < [K^+]_i$ because the ions need to enter the small channel, which reduces their concentration. A simple model considers that $C(0) = \beta [K^+]_i$, where β is called the *partition coefficient* (Hille, 2001). However, this subtlety does not change the result in our case.

Remarkably, in the case of a membrane permeable to a single ion species, we do not need to know how exactly the electrical field depends on the distribution of charges. We only need to know the effect of the electrical field on the movement of charges. In an electrolyte, the average velocity of a charge is proportional to the field:

$$\mathbf{v} = \pm \mu \mathbf{E}$$

where μ is called the *mobility* of the ion, and is expressed in m².V⁻¹.s⁻¹. The velocity is in the direction of the field for positive charges, and in the opposite direction for negative charges. This velocity is only an average, because ions actually move in all directions because of thermal agitation. This is why it is often called *drift velocity*, it is the bias in velocity induced by the field, and it is in general orders of magnitude smaller than the actual instantaneous velocity of a given ion. We can then add the effect of the electrical field (the bias) to the effect of diffusion calculated above. In our one-dimensional channel with K⁺ ions, the net flux of ions due to the electrical field is proportional to the drift velocity and to the ion concentration:

$$J_E(x) = \mu E(x).C(x)$$

If we were dealing with an anion (e.g. Cl^-), we would insert a minus sign. The electrical field has the property that it can be expressed as the gradient of a scalar quantity, and we call that quantity the electric potential (or more precisely, its opposite):

$$\mathbf{E} = -\mathbf{grad} \ V = -(\frac{\partial V}{\partial x}, \frac{\partial V}{\partial y}, \frac{\partial V}{\partial z})$$

In our context, the potential only varies in the x dimension, and the ion flux due to the electrical field is then:

$$J_E(x) = -\mu C \frac{\partial V}{\partial x}$$

The equilibrium potential

Therefore, the total ion flux, combining the effects of diffusion and electrical field, is:

$$J = J_D + J_E = -D\frac{\partial C}{\partial x} - \mu C\frac{\partial V}{\partial x}$$

In this formula, there are two parameters that are specific of the ion species: the diffusion parameter D and the mobility parameter μ . Because they depend on the same properties, there is actually a quantitative relation between them, which has been derived by Einstein:

$$D = \frac{RT}{|z|F}\mu$$
 (Einstein relation)

where z is the signed number of charges carried by the ion (in the present case z = 1), $R = 8.314 \text{ J.K}^{-1} \text{.mol}^{-1}$ is the universal gas constant, T is absolute temperature (in kelvin), and $F = 96485.34 \text{ C.mol}^{-1}$ is Faraday's constant.

We can now calculate the equilibrium potential, which is such that there is no net ion flux. We can see that mobility μ disappears when we set J = 0, so that we get:

$$-\frac{RT}{F}\frac{\partial C}{\partial x} - C\frac{\partial V}{\partial x} = 0$$

2.2. MEMBRANE POLARIZATION

We can rearrange this equation in the following way:

$$\frac{\partial V}{\partial x} = -\frac{RT}{F} \frac{\partial (\log C)}{\partial x}$$

We then integrate between the two sides of the channel:

$$V(L) - V(0) = -\frac{RT}{F} \log \frac{C(L)}{C(0)}$$

Position x = 0 corresponds to the cytosol, and the membrane potential is defined as $V_m = V(0) - V(L)$. Thus we obtain:

$$V_m = \frac{RT}{F} \log \frac{[K^+]_o}{[K^+]_i}$$

This is called the Nernst potential or reversal potential for K^+ , and is usually denoted E_K . For the concentrations given on table 2.1 for the cat motoneuron, we obtain $E_K = -87$ mV at 37° C (values are given in the table for temperature $T = 20^{\circ}$ C). Our analysis can be trivially generalized to any ion species S with z charges (z can be positive or negative), and we obtain the Nernst equation for the reversal potential:

$$E_S = \frac{RT}{zF} \log \frac{[S]_o}{[S]_i}$$
(Nernst equation)

To give an order of magnitude, $RT/F \approx 26.5$ mV at 37°C, and 25 mV at 20°C. Here as well as everywhere else in this book, the logarithm is the natural logarithm (base $e \approx 2.72$), sometimes denoted ln. In base 10, Nernst equation would read

$$E_S = \frac{RT \log 10}{zF} \log_{10} \frac{[S]_o}{[S]_i} \approx 60 \text{ mV.} \log_{10} \frac{[S]_o}{[S]_i} \text{ at } T = 37^{\circ} \text{C}$$

For example, for the cat motoneuron (concentrations on table 2.1), we immediately see that $E_{Na} \approx 60 \text{ mV}$ at 37°C. This means that, if the cat motoneuron's membrane were only permeable to Na⁺, then its membrane potential would be 60 mV.

How many ions are displaced?

The Nernst equation gives the equilibrium potential in terms of the final ionic concentrations at equilibrium. In principle, these final concentrations are different from the initial concentrations. Suppose we start with two electroneutral solutions, so that the membrane potential is $V_m = 0$ mV. Then K⁺ ions flow from the cytosol to the extracellular space until equilibrium is established. At equilibrium, the two solutions are then not electroneutral anymore: there is an excess of positive charges, in the form of K⁺ ions, in the extracellular space, and a lack of K⁺ ions or equivalently an excess of negative charges, in the cytosol. How large is this displacement of ions?

Electrically, the ionic solutions are two conductors separated by a thin isolating membrane. Electrostatic theory tells us that excess charges concentrate at the boundary of a conductor, while the interior is electroneutral. This means that the excess of positive and negative charges will be seen near the outer and inner side of the membrane, respectively. Intuitively, it is clear that the electric field produced by these charges, and therefore the membrane potential, should be proportional to the number of charges. This is the electrical behavior of a *capacitor*, which is summarized in the equation Q = C.V, where Q is the charge on one side of the capacitor, V is the electric potential on that side, relative to the other side, and the proportionality constant C is called the *capacitance*, in units of farad (F). Expressed per unit area, we obtain:

$$\sigma = c_m V_m$$

where σ is the surfacic density of charge on the inner side of the membrane, and c_m is the membrane capacitance per unit area, called *specific capacitance*. The capacitance is inversely proportional to the width of the membrane, and for biological membranes is about $c_m = 1 \,\mu\text{F/cm}^2$ (possibly 0.9 $\mu\text{F/cm}^2$ in neurons (Gentet et al., 2000)).

We can now calculate the change in ion concentration necessary to establish the Nernst equilibrium. Let us consider for example a piece of axon of diameter d and length l. Its surface area is $\pi d.l$ and therefore there is an excess of charge on its inner surface equal to $\sigma \pi d.l = c_m V_m \pi dl$, which is negative. The charge of one mole of unit charges is the Faraday constant F. Therefore, the number of moles of K⁺ that are missing in the cell at equilibrium is $c_m |V_m| \pi dl/F$. The volume of this piece of axon is $\pi d^2 l/4$. Therefore, the change in intracellular K⁺ concentration necessary to establish the equilibrium is:

$$\Delta[K^+]_i = \frac{4c_m V_m}{dF}$$

For example, with $V_m = -87$ mV and $d = 1 \ \mu m$, we obtain $\Delta [K^+]_i = -0.04$ mM. Remember that $[K^+]_i$ is of order 150 mM in vertebrate neurons (table 2.1). Thus even in very thin axons, the displacement of ions required to establish the membrane polarization is very small and is not expected to impact intracellular ionic concentrations.

2.2.2 A membrane permeable to K⁺ and Cl⁻

At rest, the membrane is in fact also permeable to other ionic species than K^+ , mostly Cl^- and to a lesser extent Na⁺. In the squid giant axon, the relative membrane permeabilities to K^+ , $Cl^$ and Na⁺ are 1:0.45:0.04 (Hodgkin, 1951). Therefore, it is not unreasonable to start by assuming that the membrane is only permeable to K^+ and Cl^- . The Nernst equation applies when the net ion flux is null: $V_m = E_K$ when the flux of K^+ is null, and $V_m = E_{Cl}$ when the flux of $Cl^$ is null. Therefore, we see that an equilibrium can only exist if $E_K = E_{Cl}$. According to Nernst equation, this means that:

$$\frac{[K^+]_o}{[K^+]_i} = \frac{[Cl^-]_i}{[Cl^-]_o}$$

or equivalently

 $[K^+]_o [Cl^-]_o = [K^+]_i [Cl^-]_i$

(Gibbs-Donnan equilibrium)

This situation is called a *Gibbs-Donnan equilibrium*. This simple model was found to be quite accurate in frog muscle fibers (Boyle and Conway, 1941), but it does not apply generally. In table 2.1, we see that in the squid axon, $[K^+]_i/[K^+]_o \approx 40$ and $[Cl^-]_o/[Cl^-]_i \approx 13$. The situation is similar in the cat motoneuron. We can see that the Nernst equation cannot fully explain the resting potential of neurons, because it describes an equilibrium situation and the membrane cannot be simultaneously at equilibrium for K⁺, Cl⁻ and Na⁺, which have very different Nernst potentials. We now consider the more general case when the membrane is permeable to these three ion species.

2.2.3 A membrane permeable to K^+ , Cl^- and Na^+

Since the Nernst potentials of K^+ , Cl^- and Na^+ are different, there must be at rest a non-zero flux of ions for at least two ion species. Therefore, we must assume that there is another mechanism



Figure 2.11: Constant field theory. A, Electric field is assumed to be constant in the membrane, so that electric potential varies linearly. B, Calculated GHK current for a 10-fold ratio of extrato intracellular concentration of Na⁺ at $T = 37^{\circ}$.

that ensures the stability of ionic concentrations. We will discuss this mechanism in section 2.2.4, and simply assume for the moment that intracellular and extracellular concentrations are fixed.

In this case, the electrical equilibrium is reached not when all ionic currents are null, but when the sum of the three currents is null. We call this situation a *dynamic equilibrium*. To calculate the equilibrium membrane potential, we then need to know how each of these ionic currents depends on V_m , i.e., their *current-voltage relationship*.

The current-voltage relationship of permeable membranes

In section 2.2.1, we have shown that for a channel permeable to a single ionic species S, the ion flux and therefore the current I_S^{10} vanish when V_m equals the reversal potential (or Nernst potential) E_S of that ion species. Thus a simple model would consist in postulating that the current depends linearly on V_m around E_S (that is, we linearize the current-voltage relation around E_S):

$$I_S = g_S(V_m - E_S)$$

where g_S is homogeneous to a conductance (in siemens, S). Here we have adopted the convention of seeing I_S as an outward current (directed towards the extracellular space), that is, $I_S > 0$ when positive charges leave the cell. This is an ohmic model because it is electrically equivalent to a resistor of conductance g_S (or resistance $R_S = 1/g_S$), which follows Ohm's law, in series with a battery E_S . This model appears in the Hodgkin-Huxley model of the squid giant axon (Hodgkin and Huxley, 1952).

Unfortunately, current-voltage relationships are generally not linear, even when membrane permeability is fixed (remember that we only consider "passive" properties of membranes in this chapter, i.e., with no voltage-dependent changes in the configuration of ionic channels). In a number of cases this relation shows *rectification*, meaning that current passes more easily in one

¹⁰Current I is related to flux J by the equation I = zF.A.J, where z is the (signed) number of charges carried by one ion, F is the charge of a mole of ions and A is the section area of the channel.

direction than in the other one. Rectification occurs especially when ion concentrations on the two sides of the membrane are very different. It has been shown by Goldman using a variety of artificial membranes, who also proposed a simple model of the current-voltage relation (Goldman, 1943). The theory was then applied to neuron membranes by Hodgkin and Katz (1949). It is based on the postulate that the electrical field $\mathbf{E}(x)$ is constant across the membrane (Figure 2.11A), and that ions of different species do not interact with each other. For this reason, Goldman's theory is often called *constant field theory*. The membrane *permeability* P_S to ion species S is defined as the ion flux divided by the concentration gradient across the membrane in the absence of an applied electrical field¹¹:

$$J_D = -P_S([S]_o - [S]_i)$$

With the same approach as in section 2.2.1, we find that the current carried by these ions per unit of membrane area (in A/m^2) equals:

$$I_{S} = P_{S} z_{S}^{2} \frac{V_{m} F^{2}}{RT} \left(\frac{1}{1 - \exp(-z_{S} V_{m} F/RT)} [S]_{i} + \frac{1}{1 - \exp(z_{S} V_{m} F/RT)} [S]_{o} \right)$$
(GHK current equation)

where z_S is the signed number of charges carried by ion species S. A demonstration can be found in (Hille, 2001; Tuckwell, 1988). This equation consists of two terms, corresponding to the influx and efflux of ions. The two terms in brackets correspond to a weighted average of $[S]_i$ and $[S]_o$, where the weights depend on V_m (indeed these two weights sum to one¹²). An example is shown on Figure 2.11B for the Na⁺ current¹³, in the case corresponding to the cat motoneuron (concentrations in table 2.1), at $T = 37^{\circ}$.

Let us first consider the case when $[S]_i = [S]_o$, so that the expression in brackets is just [S]. In this case, the Nernst potential is $E_S = 0$ mV, and the current I_S is a linear function of V_m , that is, it follows the ohmic model:

$$I_S = P_S z_S^2 \frac{F^2[S]}{RT} V_m$$

and the conductance is the factor in front of V_m . We note that this conductance is a (linear) function of [S]. Intuitively: there is more current when there are more ions in the medium. Thus it is important to realize that the conductance of a ionic channel is a property that depends on the context in which the channel functions, here ionic concentration in the medium, rather than an intrinsic property of the channel.

In the more general case, $[S]_i \neq [S]_o$, the current-voltage relation is not linear. It is possible to rewrite the GHK equation as a function of the Nernst potential E_S :

$$I_{S} = gV_{m} \frac{1 - \exp(z_{S}(E_{S} - V_{m})F/RT)}{1 - \exp(-z_{S}V_{m}F/RT)}$$

$$I_{S} = P_{S} z_{S}^{2} \frac{V_{m} F^{2}}{RT} \frac{[S]_{i} - [S]_{o} \exp(-z_{S} V_{m} F/RT)}{1 - \exp(-z_{S} V_{m} F/RT)}$$

¹¹It can be shown that ion concentration C(x) should indeed increase linearly across the membrane when there is no electrical field, according to Fick's diffusion laws.

¹²using $1/(1 + e^x) + 1/(1 + e^{-x}) = 1$.

 $^{^{13}\}mathrm{For}$ numerical reasons, the following equivalent expression was used to plot this graph:

2.2. MEMBRANE POLARIZATION

where

$$g = P_S z_S^2 \frac{F^2}{RT} [S]_i$$

has the dimension of a conductance. In this form, the current is expressed as a conductance times a driving force, as in the ohmic model, except the driving force is a nonlinear function of voltage. From this expression, we also notice that $I_S = 0$ at the Nernst potential, as expected.

For $V_m = 0$ mV, the definition of membrane permeability can be recovered from the GHK current equation (technically, one must calculate a Taylor expansion of the exponential):

$$I_S = P_S z_S F([S]_i - [S]_o)$$

At large positive and negative potential, $I_S(V_m)$ is asymptotically linear. Let us assume that $z_S > 0$, i.e., ion S carries a positive charge. Then at hyperpolarized voltages $(V_m \ll 0)$:

$$I_S \approx P_S z_S^2 \frac{F^2[S]_o}{RT} V_m$$

and conversely at depolarized voltages:

$$I_S \approx P_S z_S^2 \frac{F^2[S]_i}{RT} V_m$$

In both cases, the current follows an ohmic model, but the conductance depends on the direction of the current. This behavior is called *rectification*. Using this current-voltage relationship, we can now calculate the resting potential when the membrane is permeable to several ion species.

The Goldman-Hodgkin-Katz voltage equation

What is the equilibrium potential in the GHK model? First, when there is a single ion species, we recover the Nernst potential by setting $I_S = 0$. If we consider several ion species, for example Na⁺, K⁺ and Cl⁻, then setting $I_{Na^+} + I_{K^+} + I_{Cl^-} = 0$ gives the following equilibrium potential:

$$V_m = \frac{RT}{F} \log \frac{P_{Na}[Na^+]_o + P_K[K^+]_o + P_{Cl^-}[Cl^-]_i}{P_{Na}[Na^+]_i + P_K[K^+]_i + P_{Cl^-}[Cl^-]_o}$$
(GHK voltage equation)

The GHK voltage equation can be extended to divalent ions (i.e. Ca^{2+}) (Tuckwell, 1988). It is broadly used to calculate resting potentials, and tends to give good results (see for example Hodgkin and Katz (1949) for the squid axon). It is quite different from the result obtained with the ohmic model of ionic currents. Indeed if ionic currents were linear functions of voltage, then the resting potential would be:

$$V_m = \frac{g_{Na} E_{Na} + g_K E_K + g_{Cl} E_{Cl}}{g_{Na} + g_K + g_{Cl}}$$

where g_S are channel conductances. But this simpler formula generally does not work as well as the GHK equation. In practice, permeabilities are not directly measured, but rather are free parameters found by fitting the GHK equation to data (see Tuckwell (1988) for the relevant methods).



Figure 2.12: Two ionic pumps: the Na⁺/K⁺-ATPase, which uses ATP as a source of energy, and the Na-K-Cl cotransporter (NKCC1, right), which uses the Na⁺ gradient as a source of energy.

Biophysical subtleties

It is important to realize that both the ohmic model and the GHK model of membrane electrodiffusion are idealized models, based on a number of assumptions. The GHK model, for example, assumes a constant field across the membrane and independence of ion movements. The first assumption could be wrong in a number of cases because of the phenomenon of *adsorption*, where molecules such as ions can adhere to the membrane by surface forces (McLaughlin, 1977). These surface charges create a discontinuity in electric potential as the layer of adsorbed ions is crossed. This phenomenon has been first postulated in squid and Xenopus nerve (Frankenhaeuser, 1960). It is also thought to occur for example in Paramecium (Eckert and Brehm, 1979), where it might be quite important as the extracellular environment is highly variable in natural environments. The GHK model can be updated to take into account this phenomenon (Frankenhaeuser, 1960; Tuckwell, 1988).

The independence hypothesis is also not accurate in general. One example is that the conductance of a single channel, measured with the patch clamp technique in symmetrical solutions, often saturates when ion concentration is increased. This and other phenomena are addressed in detail in (Hille, 2001).

2.2.4 Ion pumps

At rest, there is a constant flux of K^+ , Cl^- and Na^+ ions. This would ultimately lead to the disappearance of concentration gradients across the membrane if there were no opposing mechanism. The maintenance of concentration gradients is ensured by the activity of *ion pumps* or *ion transporters*. These are transmembrane proteins that move ions across the membrane. This movement requires energy. A number of ion pumps use the energy stored in concentration gradients across the membrane (Figure 2.12). For example, the Na-K-Cl cotransporter (NKCC1) moves Na⁺, K⁺ and Cl⁻ into the cell in a 1:1:2 ratio, which makes it electroneutral. Energy is provided by the Na⁺ gradient. The chloride potassium symporter (KCC2) expels K⁺ and Cl⁻ (also electroneutral); energy is then provided by the K⁺ gradient. It can be seen indeed on table 2.1 that $[K^+]_i/[K^+]_o > [Cl^-]_o/[Cl^-]_i$ (see section 2.2.2).

But of course this type of ion pump can only work if the concentration gradients are already established, and therefore ultimately another source of energy is required. The main source of energy in the cell is ATP (adenosine triphosphate). It provides energy in particular to a ionic pump called Na⁺/K⁺-ATPase: for one molecule of ATP, 3 Na⁺ ions are moved out while 2 K⁺ ions are moved in, both against their concentration gradients. The activity of this ion pump contributes a large part of the energy spent by neurons (Attwell and Laughlin, 2001; Howarth et al., 2012). We notice that one net positive charge leaves the cell for every ATP molecule. Therefore, the activity of the pump creates an outward (hyperpolarizing) current. The pump is said to be *electrogenic*, and therefore it impacts the resting potential. The GHK voltage equation can be modified to incorporate this effect (Tuckwell, 1988).

There is another electrogenic pump in neurons, the sodium-calcium exchanger, which expels one Ca^{2+} ion from the cell in exchange of three Na⁺ ions moved into the cell. It uses the Na⁺ gradient as a source of energy. Another important pump is the plasma membrane Ca^{2+} ATPase (PMCA), which uses ATP to move one Ca^{2+} ion in and two H⁺ ions out (Thomas, 2009).

These considerations will be important when we discuss the energetic consumption of neurons in chapter ??.

2.3 Osmosis and mechanics

Electrophysiological theory traditionally focusses on movements of ions. Here we will give a brief overview of two other biophysical phenomena that are in principle relevant to the matter of this book: movement of water and movement of membrane. The rest of this book will largely ignore them, but it is important to be aware of what models neglect.

We have noted that biological membranes are permeable to water. Osmosis is the flow of water across a semipermeable membrane into a region of higher solute concentration. It results in changes in ion concentrations and cell volume, in a way that tends to equalize solute concentrations on the two sides of the membrane. Let us examine ion concentrations for the squid giant axon in table 2.1 (p 10). If we add the concentrations of all extracellular ions, we obtain about 1 M. As we already noted, the list of intracellular ions does not include the large number of organic anions that are necessary to ensure electroneutrality. But we can deduce from the table that their concentration should be on the order of 500 mM so as to match the positive charge of cations¹⁴. Thus the total concentration of intracellular anions is also about 1 M, and the cell is in osmotic equilibrium. More precisely, osmosis depends on osmotic concentrations or osmolarity, which are slightly different from molar concentrations to account for non-idealities of solutions.

Osmosis is often described as the diffusion of water through the membrane for didactical reasons, but its physical basis is in fact quite different (Kramer and Myers, 2013). Consider the ions dissolved in the intracellular medium. When they randomly hit the membrane under the effect of thermal agitation, ions are reflected and this momentum is transmitted to the water molecules. This produces in a force exerted on the fluid, directed away from the membrane, that is, directed inward. The resulting pressure (force per unit membrane area) is given by *van't Hoff's law* (under ideal conditions):

$$P_{\text{osmotic}} = [\text{solute}]RT$$
 (van't Hoff's law)

¹⁴This is of course a rough estimate, as it depends on the number of charges per molecule.

This expression is analog to the law of ideal gases. In other words, for the calculation of pressure, a solution behaves essentially like a gas of ions. The same holds for the extracellular side, with a force directed outward. As the membrane is permeable to water, there is a flow of water per unit area equal to

$$Q = -\Delta P_{\rm osmotic}/R_{\rm flow}$$

where R_{flow} is the membrane resistance to water flow. In other words, water flows from regions of low to high ion concentrations. A detailed mathematical treatment of osmosis and its interaction with membrane electrodiffusion can be found in (Hoppensteadt and Peskin, 2004).

Thus if the extracellular medium has lower osmolarity than the intracellular medium (lower ion concentrations), then water flows in and the cell swells. As a result, intracellular osmolarity decreases and an equilibrium may be reached. If the osmolarity difference is too large, the cell may explode and die. Conversely, if extracellular osmolarity is larger, then the cell shrinks. This phenomenon is of great importance for patch-clamp electrophysiological experiments, in which a large pipette is brought to contact with the membrane and the membrane is ruptured. The liquid content of the cell is then mixed with the pipette solution, which is much larger, and membrane stability requires the pipette solution to have the same osmolarity as the extracellular medium.

Osmosis also implies that transmembrane movements of ions should result in volume changes, and therefore in changes in the concentration of all intracellular ions. This effect has indeed be observed in neurons (Tasaki et al., 1985), but it is neglected in standard electrophysiological theory. We can estimate the expected magnitude of the effect for an action potential as we have done in section 2.2.1. During the rising phase of the AP, N moles of Na⁺ ions enter the neuron. If we assume that the volume V changes by ΔV so as to preserve intracellular osmolarity O (in units of Osm/L, which is roughly equivalent to mM), then:

$$O.V + N = O.(V + \Delta V)$$

This means that the volume change due to Na⁺ entry is $\Delta V = N/O$. Suppose that the neuron is depolarized by about 100 mV. This corresponds to a net movement of charges equal to $Q = c_m A.100$ mV where c_m is the specific capacitance of the membrane (around 1 μ F/cm²) and A is membrane area. This charge corresponds to N = Q/F moles of Na⁺. Therefore, we have:

$$\frac{\Delta V}{A} = \frac{c_m.100 \text{ mV}}{O.F}$$

Looking at table 2.1, we see that the osmolarity of a cat motoneuron's intracellular medium should be around 300 Osm/L. With these numbers, we find:

$$\frac{\Delta V}{A} \approx 35 \ pm$$

For a cylinder (e.g. an axon), the ratio of volume to area is the diameter divided by 4, and therefore we would expect a change in diameter of about 140 pm (0.14 nm) at the peak of an AP. For a sphere, this ratio is diameter divided by 6, and therefore would expect a change in diameter of about 210 pm (0.21 nm). The repolarization phase of the AP would have the opposite osmotic effect on cell volume. Thus there should be a transient change in volume due to osmotic effects during an AP, but it should be tiny. Therefore it is reasonable to neglect its effect on ionic concentrations. The net change in volume after an AP should be even smaller, but not zero because even if electroneutrality is respected, ions of similar osmolarity can carry different numbers of charges and osmolarity can be slighly different for different ion species with the same valence.

2.4. SUMMARY AND EPISTEMOLOGICAL NOTES

The cell can change volume due to osmosis, but it may also change shape, i.e., it can bend. This can occur by osmosis but also by the effect of the electrical field applied to the membrane, which can change membrane curvature. This may theoretically result in the propagation of a mechanical wave along the axon, accompanying the action potential wave (El Hady and Machta, 2015). The propagation of a mechanical wave of about 2 nm amplitude has indeed been measured in a crayfish axon (Hill et al., 1977) (which has a diameter of 180 μ m), with the same velocity as the action potential. The potential importance of this phenomenon is that the properties of ionic channels can in turn be affected by membrane curvature (Anishkin et al., 2014). If the effect were substantial, then action potential propagation should be modeled as an electromechanical wave. As we will see in chapter ??, in the classic work of Hodgkin and Huxley, a biophysical model of the action potential was fitted to measurements in an isopotential squid giant axon, that is, where a metal electrode was inserted into the axon so as to make it isopotential (Hodgkin and Huxley, 1952). The model was based on electrodiffusion theory and did not include mechanical effects. When the model was extended to include electrical propagation along the axon (i.e., with axial currents), the predicted conduction velocity was found to be within 20% of the measured velocity. This suggests that electromechanical effects are not dramatic (at least in the squid giant axon), but the question is still open as to whether they might nonetheless be significant.

An introduction to the physics of cell membranes can be found in (Phillips et al., 2008).

2.4 Summary and epistemological notes

Membrane polarization is classically explained by electrodiffusion through a semi-permeable membrane, resulting from the opposing effects of diffusion and electrical forces on the movement of charged molecules (ions). At a general level, this theory has been very well tested and confirmed. Let us review the main concepts.

Ions move by diffusion through the membrane. As they carry an electrical charge, the resulting charge imbalance around the membrane creates an electrical field. In turn, the electrical field exerts a force on the ions in the opposite direction. Thus, for every ion species, there is a value of the electrical field, and therefore of the membrane potential, for which there is no net movement of ions across the membrane: we then call this value the reversal potential. The reversal potential equals the Nernst potential when the membrane is permeable to a single ion species (p 15). It is also the Nernst potential when the membrane is permeable to several ion species, for each of the ion species, under the assumptions of Goldman-Hodgkin-Katz (GHK) theory (constant field and independence of ions, p 18). We have noted cases in which GHK theory must be amended. When the membrane is permeable to several ion species with different reversal potentials, then the equilibrium potential or resting potential lies somewhere between these reversal potentials. A theoretical prediction for that value is provided by GHK theory, which is fairly successful (p 19). At equilibrium, there must then be non-zero ion fluxes that balance each other, and therefore intracellular concentrations would be expected to change and ultimately concentration gradients across the membrane should disappear. Stable ionic concentrations are maintained by ionic pumps, and this requires external energy, provided in the form of ATP.

Beyond these important general points, the situation is rather complex. As we will see in the next chapter, ionic currents are often presented as following the ohmic model, i.e., as a battery representing the Nernst potential in series with a resistor representing the "conductance" of the channel. This should be seen not as a biophysical fact but rather as a convenient simple model. We will largely use this simple model in the rest of this book, but it should kept in mind that the ohmic model can be inaccurate and misleading. It can be inaccurate because in many cases the current-voltage relationship is not linear even if we only consider so-called "passive" properties

of membranes, that is, with no change in permeability (no change in channel conformation). It can be misleading because even when the current-voltage relationship is approximately linear, the quantity that we call "channel conductance" is not a physical characteristic determined by membrane permeability alone, but it depends also on ionic concentrations (as appears clearly in the expression of the GHK current p 18).

This brings us to an important epistemological point, which is that there are no observations or measurements independent of theory. When we say that we measure the conductance of a channel, or of the membrane, what we really mean is that we assume that the membrane follows the ohmic model (we might check whether the current-voltage relationship is linear enough), and then we divide current by driving force $(V - E_S)$ and call the result conductance. In the same way, when we say that we measure the permeability of the membrane to various ions, what we really mean is that we fit the GHK equation to current-voltage data and call the best fitting values the membrane permeabilities. This is true also of seemingly trivial concepts, for example the membrane potential. The way the membrane potential is typically measured is by inserting an electrode into a cell and measuring the voltage difference between that electrode and a reference electrode at a fixed position far from the cell. What we actually measure then is rather the intracellular potential V_i , but we call it the membrane potential $V_m = V_i - V_e$ because we assume that the extracellular medium is isopotential, or at least that the potential difference between the reference electrode and the extracellular space next to the cell is constant, which clearly is an approximation.

A very important point then, is that models can be wrong in two very different ways. One trivial way is lack of precision: the model depends on a number of parameters (e.g. conductances) and the parameter values can only be known empirically with limited precision. We might call this metric uncertainty. But there is a deeper way in which a model can be wrong: a model is a particular choice of abstraction and it always captures a limited part of a complex system. For example, we have presented the standard electrodiffusion theory of membranes, but it might be that electromechanical effects turn out to be important for some questions. If this is true, then we will need different models and different types of measurements (membrane elasticity, cytoskeleton properties...), not just more electrophysiological measurements. This is not metric uncertainty but rather *epistemic uncertainty*, a much more important (and interesting) type of uncertainty. Thus, a model does not necessarily become more realistic because it is based on more measurements, or has more variables. Because of these two sources of model uncertainty, theoretical analysis is most powerful when it is abstract, that is, when it applies not to a particular model but to an entire class of models. In this sense, it is very different from simulation, which applies to a particular model. We will try to keep this notion in mind when we discuss excitability and other topics.

In the end, what is a model of a natural system? A model consists of three key ingredients (see Rosen (1985) for an excellent epistemological discussion of models). First, a mapping from the system to a set of values, which we call "measurements". Second, a set of possible actions on this system, which we call "experiments". Third, a set of postulated relationships between measurements, and between actions and measurements. We never have direct access to a natural system, and the same system can have many models. An accurate model is a model for which the postulated relationships are accurately verified; but a good model also has the quality that it applies to a large number of relationships, i.e., it has a large *empirical content* (see Brette (2015) for a discussion of these concepts in the context of neuron models).

With these general notions in mind, we will now turn to the biophysical basis of action potentials.

Bibliography

- Anishkin, A., Loukin, S. H., Teng, J., and Kung, C. (2014). Feeling the hidden mechanical forces in lipid bilayer is an original sense. Proceedings of the National Academy of Sciences, 111(22):7898–7905.
- Attwell, D. and Laughlin, S. B. (2001). An Energy Budget for Signaling in the Grey Matter of the Brain. Journal of Cerebral Blood Flow & Metabolism, 21:1133–1145.
- Boyle, P. J. and Conway, E. J. (1941). Potassium accumulation in muscle and associated changes. The Journal of Physiology, 100(1):1–63.
- Brette, R. (2015). What is the most realistic single-compartment model of spike initiation? *PLoS* computational biology, 11(4):e1004114.
- Eccles, J. C. (1957). The physiology of nerve cells.
- Eckert, R. and Brehm, P. (1979). Ionic mechanisms of excitation in Paramecium. Annual Review of Biophysics and Bioengineering, 8:353–383.
- El Hady, A. and Machta, B. B. (2015). Mechanical surface waves accompany action potential propagation. *Nature Communications*, 6:6697.
- Faisal, A. A., White, J. A., and Laughlin, S. B. (2005). Ion-Channel Noise Places Limits on the Miniaturization of the Brains Wiring. *Current Biology*, 15(12):1143–1149.
- Frankenhaeuser, B. (1960). Sodium permeability in toad nerve and in squid nerve. The Journal of Physiology, 152(1):159–166.
- Gentet, L. J., Stuart, G. J., and Clements, J. D. (2000). Direct measurement of specific membrane capacitance in neurons. *Biophysical Journal*, 79(1):314–320.
- Gentet, L. J. and Williams, S. R. (2007). Dopamine gates action potential backpropagation in midbrain dopaminergic neurons. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 27(8):1892–1901.
- Goldman, D. E. (1943). Potential, Impedance, and Rectification in Membranes. The Journal of General Physiology, 27(1):37–60.
- Herndon, R. M. (1963). The fine structure of the Purkinje cell. The Journal of Cell Biology, 18(1):167– 180.
- Hill, B. C., Schubert, E. D., Nokes, M. A., and Michelson, R. P. (1977). Laser interferometer measurement of changes in crayfish axon diameter concurrent with action potential. *Science*, 196(4288):426–428.
- Hille, B. (2001). *Ion Channels of Excitable Membranes*. Sinauer Associates. undefined 3rd Edition Ion Channels of Excitable Membranes.

- Hodgkin, A. and Huxley, A. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol (Lond), 117:500.
- Hodgkin, A. L. (1951). The Ionic Basis of Electrical Activity in Nerve and Muscle. *Biological Reviews*, 26(4):339–409.
- Hodgkin, A. L. (1964). The conduction of the nervous impulse. C. C. Thomas.
- Hodgkin, A. L. and Katz, B. (1949). The effect of sodium ions on the electrical activity of the giant axon of the squid. *The Journal of Physiology*, 108(1):37–77.
- Hoppensteadt, F. C. and Peskin, C. (2004). Modeling and Simulation in Medicine and the Life Sciences. Springer, New York, 2nd edition edition.
- Hossain, W. A., Antic, S. D., Yang, Y., Rasband, M. N., and Morest, D. K. (2005). Where is the spike generator of the cochlear nerve? Voltage-gated sodium channels in the mouse cochlea. *The Journal* of Neuroscience: The Official Journal of the Society for Neuroscience, 25(29):6857–68.
- Howarth, C., Gleeson, P., and Attwell, D. (2012). Updated Energy Budgets for Neural Computation in the Neocortex and Cerebellum. Journal of Cerebral Blood Flow & Metabolism, 32(7):1222–1232.
- Husser, M., Stuart, G., Racca, C., and Sakmann, B. (1995). Axonal initiation and active dendritic propagation of action potentials in substantia nigra neurons. *Neuron*, 15(3):637–647.
- Jacobson, K., Sheets, E. D., and Simson, R. (1995). Revisiting the fluid mosaic model of membranes. Science (New York, N.Y.), 268(5216):1441–1442.
- Kosaka, T. (1980). The axon initial segment as a synaptic site: Ultrastructure and synaptology of the initial segment of the pyramidal cell in the rat hippocampus (CA3 region). *Journal of Neurocytology*, 9(6):861–882.
- Kramer, E. M. and Myers, D. R. (2013). Osmosis is not driven by water dilution. Trends in Plant Science, 18(4):195–197.
- Kushmerick, M. J. and Podolsky, R. J. (1969). Ionic Mobility in Muscle Cells. Science, 166(3910):1297– 1298.
- Leterrier, C., Potier, J., Caillol, G., Debarnot, C., RuedaBoroni, F., and Dargent, B. (2015). Nanoscale Architecture of the Axon Initial Segment Reveals an Organized and Robust Scaffold. *Cell Reports*, 13(12):2781–2793.
- McLaughlin, S. (1977). Electrostatic Potentials at Membrane-Solution Interfaces. In Kleinzeller, F. B. a. A., editor, *Current Topics in Membranes and Transport*, volume 9, pages 71–144. Academic Press.
- Nakada, C., Ritchie, K., Oba, Y., Nakamura, M., Hotta, Y., Iino, R., Kasai, R. S., Yamaguchi, K., Fujiwara, T., and Kusumi, A. (2003). Accumulation of anchored proteins forms membrane diffusion barriers during neuronal polarization. *Nature Cell Biology*, 5(7):626–632.
- Perge, J. A., Niven, J. E., Mugnaini, E., Balasubramanian, V., and Sterling, P. (2012). Why Do Axons Differ in Caliber? *The Journal of Neuroscience*, 32(2):626–638.
- Phillips, R., Kondev, J., and Theriot, J. (2008). Physical Biology of the Cell. Garland Science, New York, 1 edition edition.
- Ramon-y Cajal, S. (1899). Texture of the Nervous System of Man and the Vertebrates.
- Rosen, R. (1985). Anticipatory Systems: Philosophical, Mathematical and Methodological Foundations. Pergamon Pr, Oxford u.a., 1st edition edition.

- Singer, S. J. and Nicolson, G. L. (1972). The Fluid Mosaic Model of the Structure of Cell Membranes. Science, 175(4023):720–731.
- Stuart, G. J. and Sakmann, B. (1994). Active propagation of somatic action potentials into neocortical pyramidal cell dendrites. *Nature*, 367(6458):69–72.
- Tasaki, I., Nakaye, T., and Byrne, P. M. (1985). Rapid swelling of neurons during synaptic transmission in the bullfrog sympathetic ganglion. *Brain Research*, 331(2):363–365.
- Thomas, R. C. (2009). The plasma membrane calcium ATPase (PMCA) of neurones is electroneutral and exchanges 2 H+ for each Ca2+ or Ba2+ ion extruded. *The Journal of Physiology*, 587(2):315–327.
- Thome, C., Kelly, T., Yanez, A., Schultz, C., Engelhardt, M., Cambridge, S., Both, M., Draguhn, A., Beck, H., and Egorov, A. (2014). Axon-Carrying Dendrites Convey Privileged Synaptic Input in Hippocampal Neurons. *Neuron*, 83(6):1418–1430.
- Tuckwell, H. (1988). Introduction to theoretical neurobiology, vol 1: linear cable theory and dendritic structure. Cambridge University Press, Cambridge. undefined Introduction to theoretical neurobiology, vol 1: linear cable theory and dendritic structure 3 NOT IN FILE.
- Verkman, A. S. (2002). Solute and macromolecule diffusion in cellular aqueous compartments. Trends in Biochemical Sciences, 27(1):27–33.
- Xu, K., Zhong, G., and Zhuang, X. (2013). Actin, Spectrin, and Associated Proteins Form a Periodic Cytoskeletal Structure in Axons. *Science*, 339(6118):452–456.

BIBLIOGRAPHY

List of Figures

2.1	Purkinje cell of the human cerebellum
2.2	Pyramidal cortical neurons
2.3	Neurons of the motor pathway 4
2.4	Neurons of the retina
2.5	Earthworm motoneuron contacted by a sensory neuron
2.6	The fluid mosaic model of membranes
2.7	Axon cytoskeleton
2.8	Soma of a Purkinje cell
2.9	A simple chemical model of a cell
2.10	Ionic diffusion through a membrane channel
2.11	Constant field theory
2.12	Ionic pumps

Index

F (Faraday constant), 14 R (universal gas constant), 14

Adsorption, 20 Anions, 10 ATP, 21 Axon diameter, 1

Capacitance (membrane), 15, 16 Cations, 10 Conductance (channel), 17 Constant field theory, 18 Cytoplasm, 7 Cytoskeleton, 7 Cytosol, 7

Dendrites, 1 Diffusion, 12 Diffusion coefficient, 13

Einstein relation (diffusion), 14 Electrical field, 13 Electrogenic, 21 Electromechanical effects, 23 Extracellular space, 10

Fick's first law, 13 Fluid mosaic model, 7

Gibbs-Donnan equilibrium, 16 Glia, 10 Goldman-Hodgkin-Katz equation, 16

Ionic composition, 10

Membrane diffusion, 7 Microtubules, 7 mM (millimolar), 10 Mobility (ion), 14 Motoneuron, 10 mRNA, 7

Na⁺/K⁺-ATPase, 21 Nernst potential, 15 Neuron size, 1 Ohmic model (channel), 17 Oligodendrocyte, 11 Organelles, 7 Osmosis, 21

Permeability, 18 Pumps, 20 Purkinje cell, 1 Pyramidal cell, 3

Rectification, 17

Spines, 3 Surface charges, 20

Transcription, 7

van't Hoff's law, 22