Theory of action potentials

Romain Brette

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Contents

Chapter 1

Action potentials

1.1 A brief history of excitability theories

1.1.1 The discovery of animal electricity

The discovery of animal electricity goes back to the experiments of Luigi Galvani in the 18th century (Galvani, 1791; Whittaker, 1910). He observed that the frog muscle contracted when a metal wire connected to the muscle was struck by lightning (Fig. 1.1A). The same contraction occurred when the leg nerve and the muscle were simply connected through a metal conductor, or when the nerve was directly brought into contact with a skinned part of the muscle. He concluded that "animal electricity" was present in the nerve and that the contraction was induced by the flow of electricity through the conductor. But Volta disagreed, he thought that the origin of electricity was actually not in the nerve but was due to the dissimilarity of materials brought to contact (Schuetze, 1983). He managed to convince his peers by building the first chemical battery, and the concept of animal electricity was abandoned for a few decades.

It was revived around 1840, when the presence of animal electricity was demonstrated by Carlo Matteucci. He directly measured a current flowing between the axial cut of a muscle and the undamaged surface using a galvanometer, the "injury current". This finding was then replicated by Emil du Bois-Reymond, who also observed that the current decreased when the nerve was electrically stimulated so as to produce muscle contraction (Fig. 1.1B). He also observed the same phenomenon in the nerve. In 1868, his student Julius Bernstein designed an ingenious device called the "differential rheotome" (Bernstein, 1868), which he used to measure the time course of the current (Fig. 1.1C; see (Verkhratsky et al., 2006; Brette and Destexhe, 2012) for a historical overview of electrophysiological techniques).

1.1.2 Bernstein's membrane theory

Building on the development of electrochemistry, in particular by Nernst, Bernstein proposed a comprehensive theory to explain animal electricity in the beginning of the 20th century, the *membrane theory* (Bernstein, 1912; Seyfarth, 2006). He proposed that the liquid inside a cell, such as a nerve fiber or muscle fiber, is a solution with a high concentration of potassium ions (K^+) (Fig. 1.2A). We will call this solution the *intracellular medium* . The solution outside the cell, which we will call the *extracellular medium* , is essentially salty water, that is, it contains mainly sodium (Na^+) and chloride (Cl^-) ions, and relatively few K^+ ions. Bernstein hypothesized that the membrane is permeable to potassium. By diffusion, K^+ ions flow from the intracellular

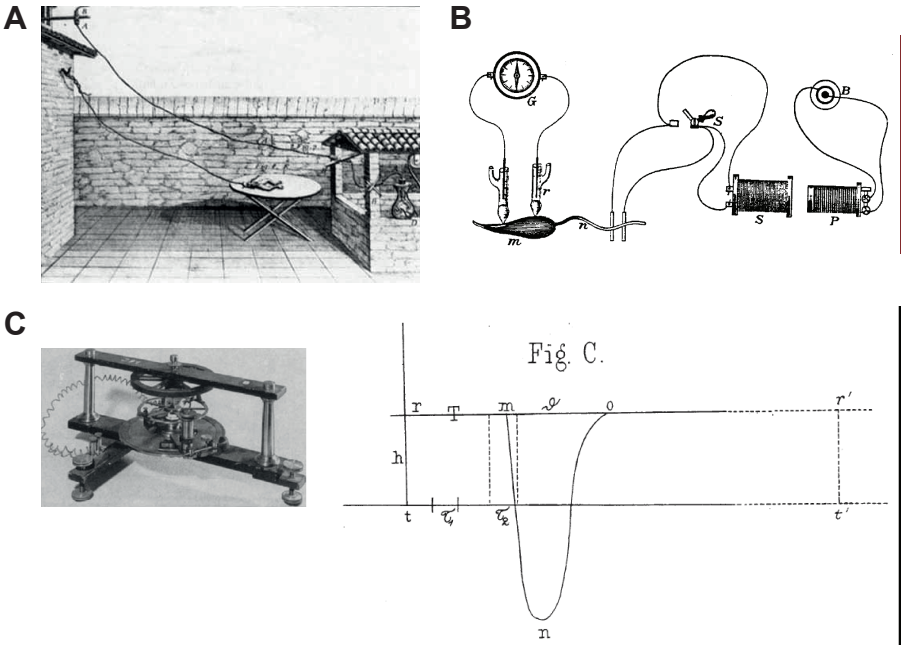

Figure 1.1: Early techniques of the 18$^{\text{th}}$ and 19$^{\text{th}}$ centuries demonstrating action potentials in frog muscle and nerve. A, Galvani's experiment where a frog's muscle contracts when the metal wire is struck by lightning (Galvani, 1791). B, Du Bois-Reymond's experimental device to measure currents in muscle and nerve. C, Bernstein's differential rheotome used to measure the time course of the current (Bernstein, 1868).

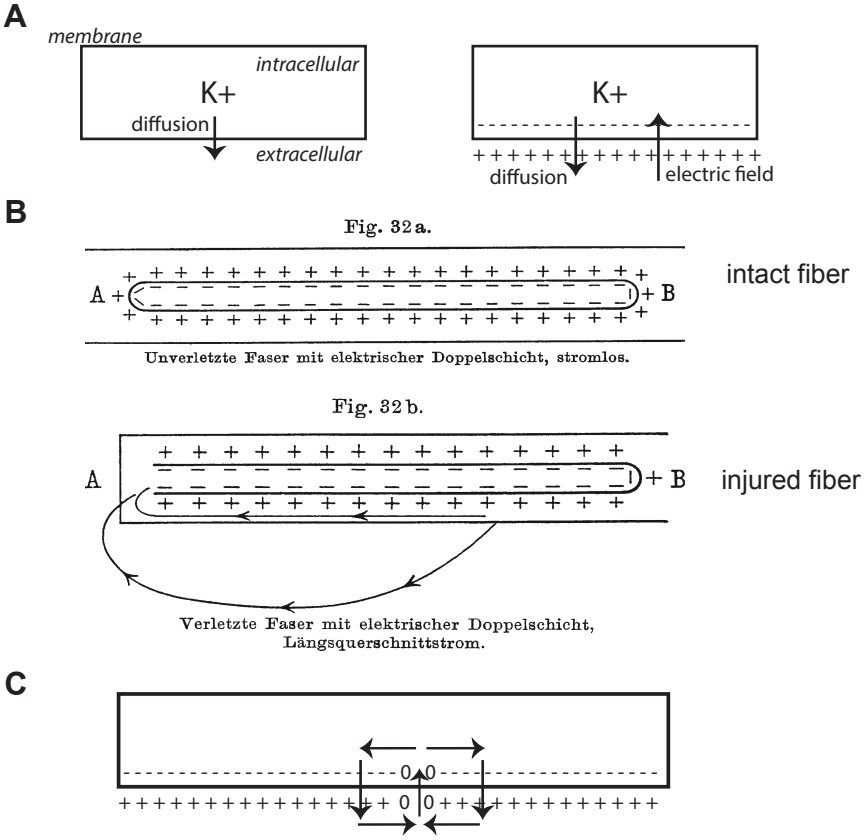

Figure 1.2: Membrane theory. A, Membrane polarization explained by electrodiffusion. B, Bernstein's explanation of the injury current (Bernstein, 1912). C, Propagation of the action potential by local currents through the intra- and extracellular and electrolytes.

medium, where they are highly concentrated, to the extracellular medium, where they are less concentrated. Since these ions carry a positive charge, this movement creates an excess of positive charges on the extracellular side of the membrane (Fig. 1.2A, right). Charges of the same sign repel each other, so this electrical force, which we call the *electric field* across the membrane, opposes further movement of ions by diffusion. A very small displacement of K^+ ions is sufficient to create an electric field that completely opposes diffusion, a situation we might call the electrochemical equilibrium. These are the basic principles of electrodiffusion , which will be exposed in chapter **??**.

Thus at equilibrium, there is an electric field across the membrane directed inwards. The mathematical properties of the electric field $\mathbf{E}$ (a vector) are such that it can be written as $\mathbf{E} = -\nabla V$ (gradient of V), where V is a scalar variable that we call the electric potential. Thus, the electric potential is higher outside than inside the cell. The *membrane potential* is defined as this difference: $V_m = V_i - V_e$ (V_i: potential inside the cell; V_e: potential outside the cell), and its value at electrochemical equilibrium, called the *resting potential* , is negative. The membrane is then said to be *polarized*.

Physically, the membrane potential is the energy that would be required to move a unit positive charge through the electric field across the membrane into the cell (considering only electric forces). The success of this theory has been rather spectacular, as it has been found to apply to all cells of living organisms, not only neurons. Indeed, the membrane of all cells is permeable mostly to potassium (but not only), all cells of living organisms are polarized, and their *resting potential* is negative: from around -200 mV in plants (Sibaoka, 1962) to about -10 mV in human red blood cells (Zavodnik et al., 1997); typically -70 to -80 mV in adult vertebrate neurons (see Binggeli and Weinstein (1986) for a more exhaustive list).

Bernstein's membrane theory also addresses the injury current (Fig. 1.2B) . When a fiber is cut, an electrical current flows from the extracellular side of the membrane, positively charged, to the axial cut of the fiber, negatively charged. This is why when Galvani put a cut nerve into contact with a muscle, he observed a contraction.

What could explain the reduction in current that Bernstein and his predecessors observed when they stimulated the nerve? As shown in Fig. 1.1C, the current decreases, and then goes back to its initial value. In Bernstein's theory, the decrease in current must correspond to a decrease in the polarization of the membrane, a *depolarization* , followed by a *repolarization* . These two terms are still used today, even though they are not completely accurate, as we will see below. Why would the membrane become less polarized? Quite naturally, Bernstein proposed that the depolarization was due to a non-specific increase in membrane permeability, the membrane "breakdown" (as if holes opened), so the solutions on the two sides of the membrane would equilibrate and the membrane potential would drop to zero. This transient change in membrane potential is what we call the *action potential* (AP), an electric potential that causes an action (here a muscle twitch).

Helmholtz had found that the AP travelled along the frog's nerve at about 27 m/s. How does the AP travel along the nerve? In Bernstein's theory, depolarization at one point of the fiber should spread to neighboring points through local current circuits (Fig. 1.2C): a current flows intracellularly from the depolarized region (marked 0 on Fig. 1.2C) to a neighboring polarized region, and must return through a path that crosses the membrane and passes through the extracellular medium. It is hypothesized that the neighboring membrane then becomes permeable, and excitation progressively propagates in this way.

This theory was highly influential and many (but not all) of these aspects turned out to be correct.

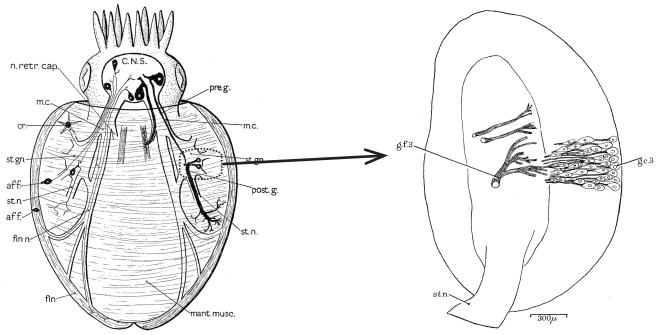

Figure 1.3: The nervous system of cephalopods (here Sepia) showing the giant axon (post. g. and g.f. 3), a syncytium formed by the fusion of many cells (st. gn. and g.c. 3) (Young, 1936).

1.1.3 The squid giant axon

In the 1930s, the giant axon of cephalopods, in particular squids, became a model of choice for electrophysiology because its diameter can reach up to 1 mm, which facilitates experimental access. That axon is quite peculiar as it is actually a syncytium, that is, a multinucleated cell formed by the fusion of hundreds of cells (Young, 1936) (Fig. 1.3). This point is worth noting, because it emphasizes the fact that these early studies were not about how APs normally initiate with natural stimuli (synaptic input), but rather about the biophysical basis of excitability and the propagation of APs along the axon (see Brette (2015) for a discussion of the Hodgkin-Huxley model as a model of AP initiation).

Thanks to the large diameter of those axons, a number of scientists began to record APs with electrodes and amplifiers. In 1939, Bernstein's membrane theory was tested and challenged by several studies in the squid giant axon. Cole and Curtis (1939) designed a clever experiment using extracellular electrodes and found that the membrane resistance dropped during the action potential, as predicted by Bernstein's theory (Fig. 1.4A). Hodgkin (1939) confirmed another prediction of Bernstein's theory, on the spreading of the AP through local currents (Fig. 1.4B). He increased the extracellular resistance by putting part of the axon in oil (for a crab) or moist air (for a squid), and found that conduction velocity decreased, as predicted. Finally, in 1939, Hodgkin and Huxley managed to insert a glass microelectrode into a squid axon and made the first single-trial [1] intracellular recording of an AP in an animal cell [2] (Hodgkin and Huxley, 1939) (Fig. 1.4C). The recording confirmed some of Bernstein's findings: the membrane is first depolarized, and then repolarized. However, it also showed very clearly that the membrane potential becomes significantly positive during the AP (about 40 mV in Fig. 1.4C), contradicting Bernstein's theory. This is sometimes called the "overshoot" of the AP. Thus during the so-called "depolarization" phase, membrane polarization decreases, reverses and then increases in the positive direction. Therefore the terms depolarization and repolarization are not entirely accurate descriptions of the two phases of the AP, but the terms are still used. Depolarization is now used to mean that the membrane potential increases, and repolarization or hyperpolarization that it decreases.

It seems that Bernstein had already noticed the overshoot but ignored it, perhaps because

[1] Bernstein's rheotome used a sample-and-hold device with fixed interval between the stimulus and the recording, and therefore required repetitions to obtain a complete recording.

[2] The first intracellular recording of an AP in a mammalian neuron was done a few years later in cat motoneurons (Brock et al., 1952).

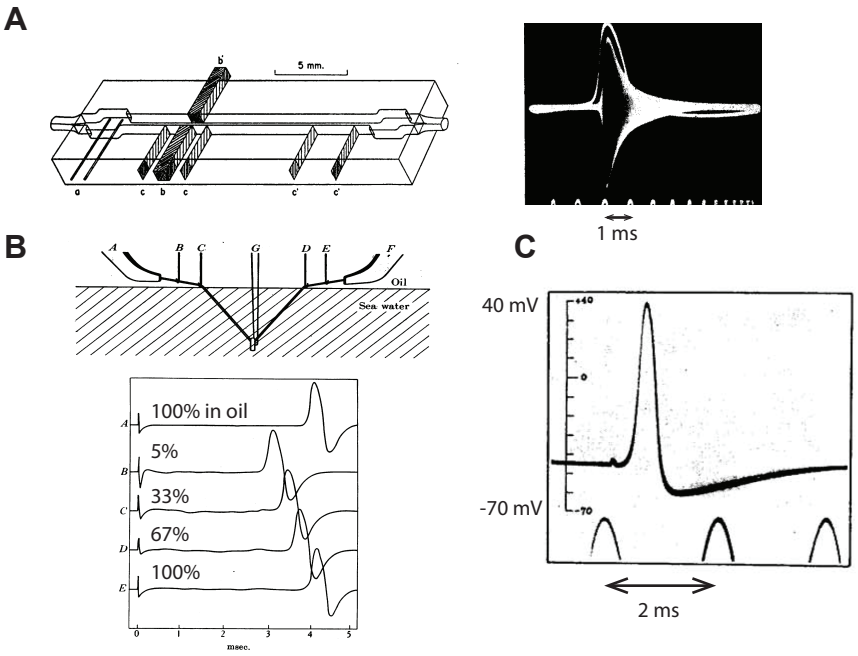

Figure 1.4: The 1939 experiments testing membrane theory. A, Membrane conductance is shown to increase dramatically during the squid axon's AP (Cole and Curtis, 1939). B, Conduction velocity of the crab's AP decreases when the axon is immersed in oil (differences in AP latency after stimulation are shown) (Hodgkin, 1939). C, Intracellular recording of an AP in the squid giant axon (Hodgkin and Huxley, 1939).

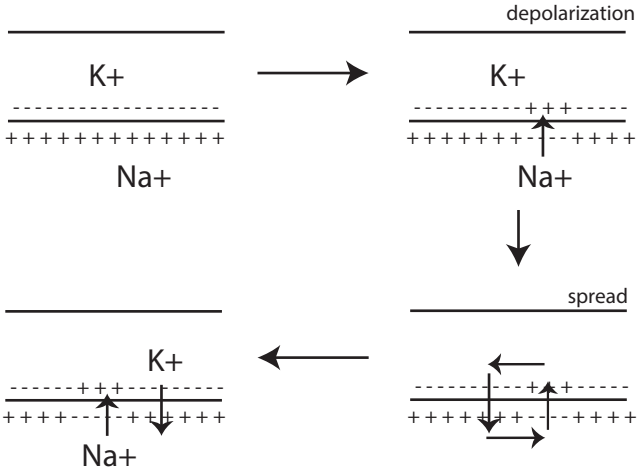

Figure 1.5: Updated membrane theory with Na^+ entry underlying the depolarization phase of the AP.

he lacked a satisfying theory to explain it and was not entirely confident in his measurements. Membrane potential cannot change sign if the membrane becomes permeable: the polarization simply disappears. Instead, Hodgkin, Huxley and colleagues demonstrated that the membrane undergoes a *selective* change in permeability, namely it becomes permeable to Na^+ ions. Because those ions are highly concentrated in the extracellular medium, they enter the axon, which reverses membrane polarization. This selective change in permeability is due to the presence of ionic channels in the membrane. Those channels are mostly closed at rest, but they open when the membrane potential increases (depolarization), letting Na^+ ions specifically enter the axon. This type of channel is called *voltage-gated* ionic channel. This influx of positive charges depolarizes the axon even more, letting more Na^+ ions enter (a positive feedback loop). The sequence of events underlying the propagation of an AP is illustrated on Fig. 1.5. At rest, the membrane is mostly permeable to K^+, so that the membrane is negatively polarized ($V_m < 0$). Some electrical stimulus depolarizes the membrane (V_m increases). As a result, the Na channels open, letting Na^+ ions enter and "depolarize" the membrane, until membrane polarization is actually switched to a positive value. The excitation then spreads through local current circuits, as described by Bernstein, which go through both the intra- and extracellular spaces. As a result, neighboring patches of membrane are depolarized, which makes Na^+ ions enter and invert membrane polarization. Meanwhile, the initial patch of membrane becomes impermeable to Na^+ because the channels "inactivate", while it becomes more permeable to K^+ ions because voltage-gated K^+ channels open (in addition to the non voltage-dependent permeability for K^+ present at rest). We will study these events in more detail in chapter **??**.

Hodgkin and Huxley demonstrated these claims by manipulation of the ionic content of the solutions combined with electrophysiology, which culminated in 1952 in the publication of a mathematical model of the AP, now called the Hodgkin-Huxley model (Hodgkin and Huxley, 1952). The model was based on measurements of voltage-gated changes in ion-specific membrane conductance, and it was fine-tuned to reproduce the recorded shape of an AP in the space-clamped squid giant axon. That is, a metal wire was inserted into the axon so as make it isopotential. This isopotential model was then extended to a spatiotemporal model of AP propagation, which turned out to correctly predict conduction velocity. Hodgkin and Huxley were awarded the Nobel prize in 1963 for their discoveries, and their model is still the basis of all biophysical neuron models today.

By that time, scientists had identified a number of distinctive features of APs:

1. APs are electrical events consisting of a large transient change in membrane polarization (typically around 100 mV).

2. APs are regenerative events triggered by membrane depolarization.

3. APs are essentially all-or-none (with some qualifications, see section 1.2): an AP is produced and propagates if the membrane is sufficiently depolarized, with similar amplitude and time course independently of stimulation strength.

4. APs are produced by voltage-dependent changes in ion-specific membrane permeability.

5. APs spread through local current circuits, passing through the membrane, intracellular and extracellular spaces.

It was postulated that the changes in membrane permeability were due to proteins in the membrane, ionic channels, which change conformation depending on membrane potential. At the scale of a single channel, the ionic flux is then discrete rather than graded: the channel is either open or closed. This theory was directly confirmed by Neher and Sakmann (two other

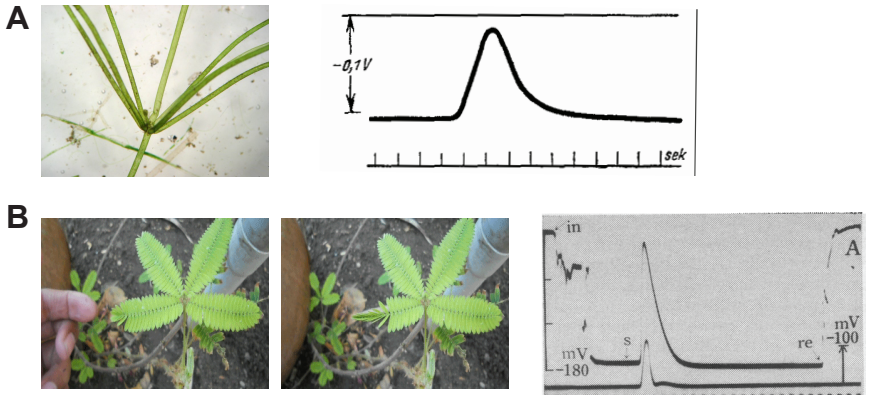

Figure 1.6: Action potentials in plants. A, Intracellularly recorded AP in Nitella a green alga (each tick is a second) (Umrath, 1930). B, Intracellular recording of an AP in Mimosa Pudica, which makes its leaves fold (Sibaoka, 1962)

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Nobel prizes) in 1976, thanks to an improved electrophysiological technique, the patch-clamp (Neher and Sakmann, 1976).

1.2 Comparative physiology of action potentials

1.2.1 Plants and fungus

Electricity was discovered in animal nerves, but it is not specific to nerves or even to animals. Not all cells produce APs, but APs have been observed across all phyla except Archea. In fact, the first recording of an AP with an intracellular microelectrode was performed by Umrath (1930) in a green alga, Nitella. As is seen on Fig. 1.6A, the amplitude and general shape of Nitella's AP is similar to the squid's AP (about 100 mV). However, as in other plants, the time scale is several orders of magnitude slower (seconds instead of milliseconds). In plants, APs often trigger protective mechanisms in response to various stimuli, such as mechanical stimulation (Wayne, 1994). Those responses are not always visible, but there are a few spectacular cases. In Venus flytrap, a carnivorous plant, mechanical stimulation by an insect triggers an AP, which in turn makes the trap close on the insect. Another famous example is Mimosa Pudica: the leaves fold inward when touched, presumably protecting them from harm or making them appear unappealing. This response is triggered by an AP (Fig. 1.6B (Sibaoka, 1962)). In addition, the AP propagates at visible pace along the stem, making neighboring leaves fold.

There are a few differences between plant APs and squid axon APs:

1. APs are orders of magnitude slower (seconds).

2. The membrane is more hyperpolarized at rest, with a resting potential around -180 mV. There is also no visible overshoot during the AP, i.e., the membrane potential does not become positive.

3. The biophysical basis of the AP is also the transmembrane movement of ions, but not the same ions. Instead of Na^+ flowing in during the rising phase, chloride (Cl^-) ions flow out

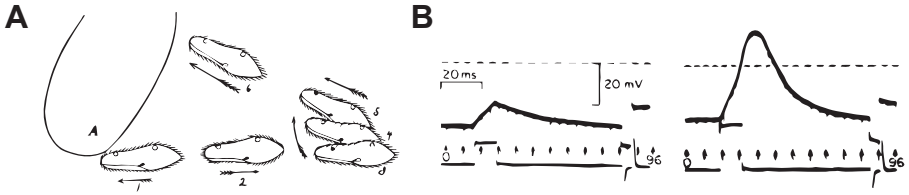

Figure 1.7: Action potential in Paramecium, a unicellular organism. A, Paramecium's avoidance reaction when it encounters an object (Jennings, 1906). B, Intracellular recording of Paramecium AP, triggered when the cell is depolarized above a threshold (Machemer and Eckert, 1973).

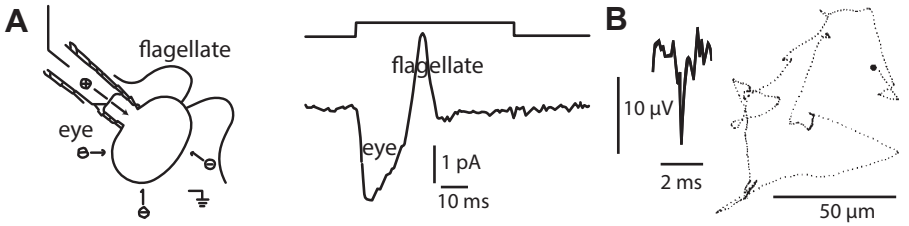

Figure 1.8: Action potentials in microscopic unicellular organisms. A, Patch-clamp recording of eye and flagellate current in Chlamydomonas, a 10 μm alga (Harz and Hegemann, 1991). B, Extracellular recording of AP (Masi et al., 2015) and trajectory (Berg and Brown, 1972) of Escherichia Coli, a 2 μm bacteria.

(and some calcium ions flow in). Repolarization is also mediated by an outflux of K^+.

Slow APs (1-2 min) have also been observed in Neurospora Crassa, a fungus (Slayman et al., 1976).

1.2.2 Unicellular organisms

Many unicellular organisms also produce APs. It has been well characterized in Paramecium, a large protozoan (100-300 μm) covered with cilia, which it uses to swim. When it stumbles on a solid object, its cilia change their beating direction and the cell swims backwards, rotates and swims forward again in a new direction (Fig. 1.7A) (Jennings, 1906). This behavior is called *avoidance reaction*. It has been shown that the avoidance reaction is triggered by an AP (Fig. 1.7B), itself triggered by various types of stimulus (reviewed in Eckert and Naitoh (1972)). The AP lasts around 20 ms, has a positive overshoot and is mediated by voltage-gated calcium channels located in the cilia (Ogura and Takahashi, 1976). It is regenerative but its amplitude depends on the stimulus. The AP elevates the intracellular concentration of Ca^{2+}, which inverts the direction of beating of the cilia.

APs have also been observed in flagellates such as Chlamydomonas (a unicellular alga of about 10 μm), which has similar swimming behavior, although the relation between behavior and electrophysiology has not been characterized in as much detail. In continuous light, Chlamydomonas swims towards or away from the light source (phototaxis), while it responds to transient light stimuli by changes in swimming direction. Light is transduced into current by a photoreceptor in the eye spot, which then triggers a calcium-mediated AP in the flagella (Fig. 1.8A) (Harz

and Hegemann, 1991). APs have also been measured in bacteria such as Escherichia Coli (Kralj et al., 2011; Masi et al., 2015), a classical model of chemotaxis commonly found in the intestine of humans, which also displays run-and-tumble behavior (Berg, 1975) (Fig. 1.8B). The relation with behavior has not been characterized at this date, but the resemblance with Paramecium and Chlamydomonas is suggestive.

Noctiluca Scintillans, a marine dinoflagellate, emits light when disturbed. The flash is triggered by an AP (Eckert and Sibaoka, 1968). The marine diatom Odontella sinensis, a unicellular photosynthetic organism, generates fast APs (on the order of the millisecond) gated by Na^+ and Ca^{2+} channels, as in animal neurons (Taylor, 2009). Actinocoryne contractilis, another protozoan with a contractile stalk, has a resting potential around -80 mV, and can produce fast APs (also on the order of the millisecond) upon electrical or mechanical stimulation (Febvre-Chevalier et al., 1986). The AP is mediated mainly by Na^+ ions and probably actively propagates between the two poles of the cell. It triggers a rapid all-or-none contraction of the stalk, mediated by Ca^{2+}.

1.2.3 Animals

Historically, the study of APs has focused on animals, such as frogs (Galvani) and cephalopods (Hodgkin and Huxley). Fig. 1.9A shows an AP recorded at the soma of a human cortical neuron (Testa-Silva et al., 2014). As in the squid giant axon, it is fast (about 1 ms), it has an amplitude of order 100 mV and a positive overshoot, depolarization is mediated by Na^+ and repolarization is mediated by K^+.

Although this is typical of neurons, there are also neurons that do not produce APs, generally sensory neurons. For example, in the cochlea, inner hair cells transduce the sound-induced vibration of the basilar membrane into a ionic current that triggers a graded release of neurotransmitter (Fig. 1.9B). These cells have no axon, and instead synapse directly with auditory nerve fibers, which produce APs. Horizontal cells, which are interneurons in the retina, also have an electrical activity and interact with other neurons but do not produce APs. They also have no axon. It was thought that retinal ganglion cells, which project to the brain, are the only retinal neurons that produce APs. However, some types of bipolar and amacrine cells also produce Na^+ APs (Heflin and Cook, 2007; Puthussery et al., 2013; Dreosti et al., 2011).

In some neurons of the auditory brainstem, APs are produced in the axon but only a strongly attenuated signal is seen in the soma (Scott et al., 2005) (Fig. 1.9C). Conversely, in cortical pyramidal neurons of mammals, APs actively propagate along dendrites, in both directions (Stuart and Sakmann, 1994). As a general rule, the spatial distribution of ionic channels that underlie the generation and propagation of APs is not homogeneous. In most adult vertebrate neurons, APs initiate not in the soma but in a small region next to the soma called the axonal initial segment (AIS), which has a high density of Na^+ channels (Coombs et al., 1957a,b; Debanne et al., 2011) (Fig. 1.10A). A similar structure has been observed in Drosophila (Trunova et al., 2011) (Fig. 1.10B), and there is electrophysiological evidence of a similar structure in neurons of Aplysia, a mollusk (Tauc, 1962). As mentioned previously, the Ca^{2+} channels that initiate APs in Paramecium are expressed in the cilia rather than in the soma. We will study the implications of this fact in chapter **??**.

The nature of APs changes during the life of a neuron. Typically, young vertebrate neurons start firing long Ca^{2+} APs (up to hundreds of ms), very early in development, before there is any synaptic contact. In the course of development, APs generally become bigger, shorter and mediated by Na^+ (Pineda and Ribera, 2007) (Fig. 1.11A). In some neurons of the auditory brainstem, APs become smaller during postnatal development (Fig. 1.9C). Some neurons also start by firing Na^+ APs (Goodman and Spitzer, 1981).

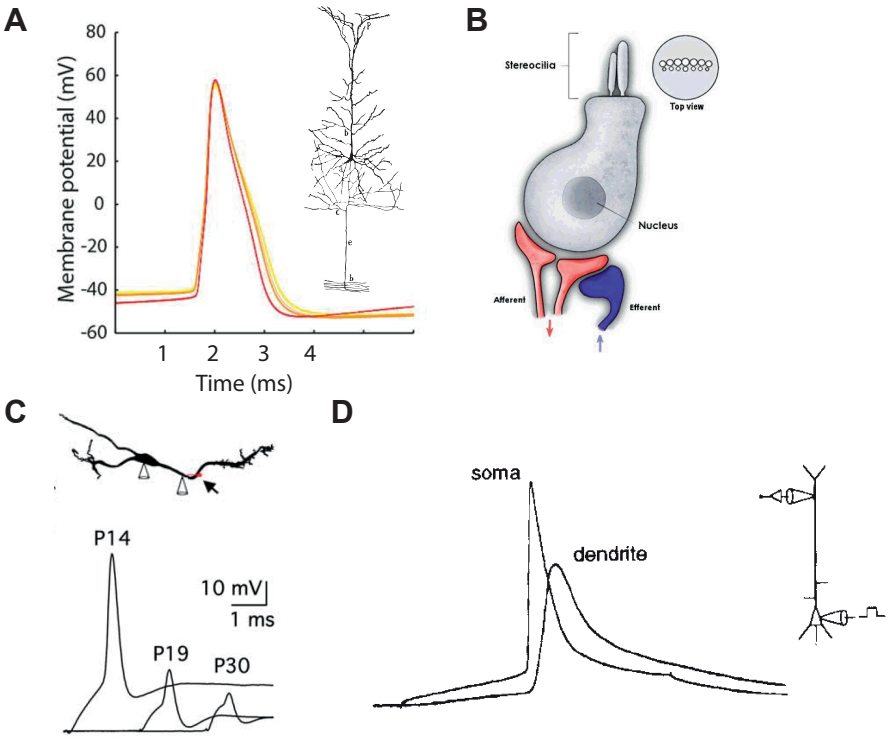

Figure 1.9: Action potentials in vertebrate neurons. A. AP in a human cortical pyramidal cell (Testa-Silva et al., 2014) (inset: cortical cell morphology in rabbit, by Ramon-y Cajal (1899)). B. Cochlear inner hair cell, which transduces sounds into current but does not produce APs (APs are produced by the afferent auditory nerve, red). C. AP in an auditory neuron of the medial superior olive at different stages of development (Scott et al., 2005). D. Backpropagation of an AP in the dendrite of a cortical neuron (Stuart and Sakmann, 1994).

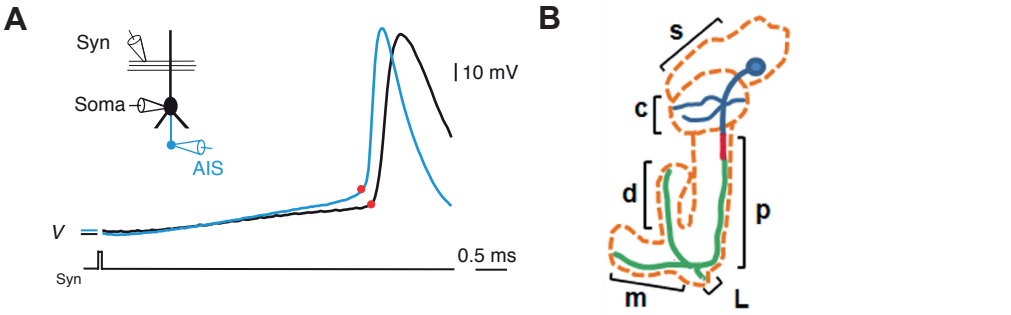

Figure 1.10: Initiation of APs in the axon. A, In cortical pyramidal cells, APs initiate in the axon initial segment, and backpropagate to the soma (Kole and Stuart, 2008). B, Neurons of Drosophila mushroom body (an olfactory structure) have a structure resembling the initial segment where APs initiate (red), close to the soma (blue) (Trunova et al., 2011).

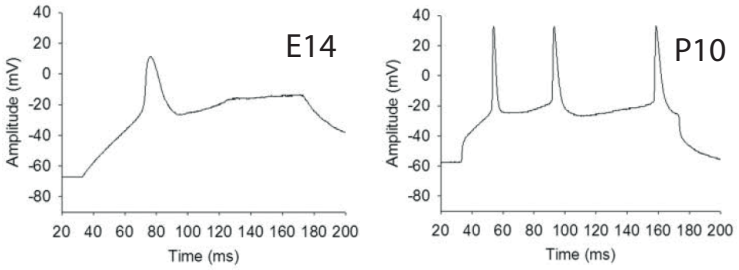

Figure 1.11: AP of a mouse cortical neuron at embryonic day 14 and postnatal day 10 (Bahrey and Moody, 2003).

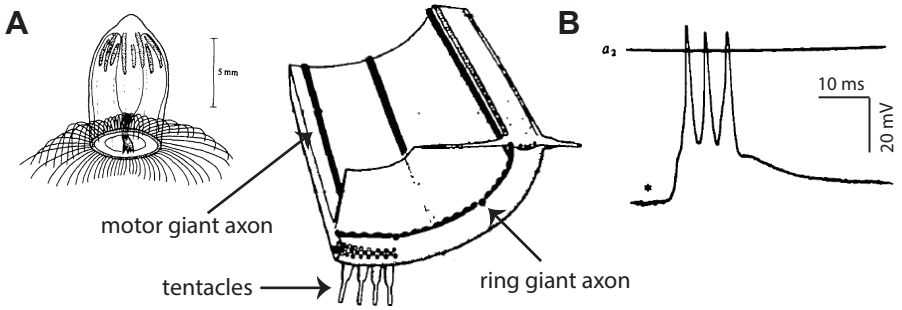

Figure 1.12: Action potentials in medusa. A, Anatomy of the escape system of Aglantha digitale, a medusa(Roberts and Mackie, 1980). B, AP in the ring giant axon.

APs also exist in animals that do not have a central nervous system. For example, jellyfish produces APs in its *nerve net*, a spatially spread set of interconnected neurons (Anderson and Schwab, 1983). In Aglantha digitale, a medusa (cnidarian), motor neurons controlling the escape reflex are synchronized by Na^+ APs produced by a giant ring axon, which runs all around the body in a closed loop (Fig. 1.12). APs have also been found in animals without any nerve. Glass sponges, for example, produce long Ca^{2+} APs (5 s) that propagate through nerveless syncytial tissues and provoke contractions (Leys et al., 1999) (Fig. 1.11B).

1.2.4 Muscles

In animals, APs are produced not only by neurons but also by muscles. Skeletal muscles of vertebrates consist of many fibers, each of which is a cell (more precisely, a syncytium) of around 50 μm of diameter and up to several tens of centimeters long (i.e., the full length of the muscle). In the 1950s, Huxley developed the *sliding filament theory* to explain the contraction of muscle fibers (Huxley, 1974): parallel filaments of actin and myosin run along the fiber, and contraction is produced by myosin sliding along actin (Fig. 1.14A). The contraction of a muscle fiber is controlled by a single motoneuron (but each motoneuron contacts several fibers), which synapses onto the fiber at the motor end plate. Each AP produced by the motoneuron triggers one Na^+ AP in the fiber, which then propagates along the fiber's membrane, called the *sarcolemma* (Fig. 1.14B). The sarcolemma is covered with little holes, which are in fact invaginations of the

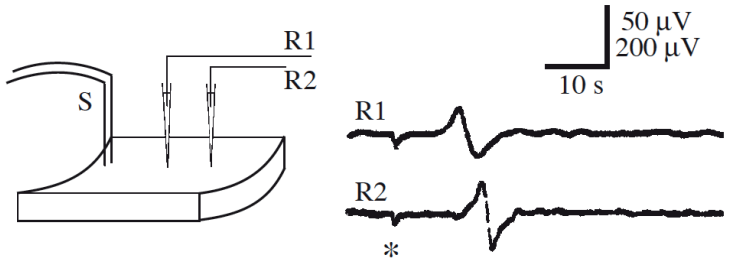

Figure 1.13: AP propagation through nerveless syncytial tissues of glass sponge (Leys et al., 1999).

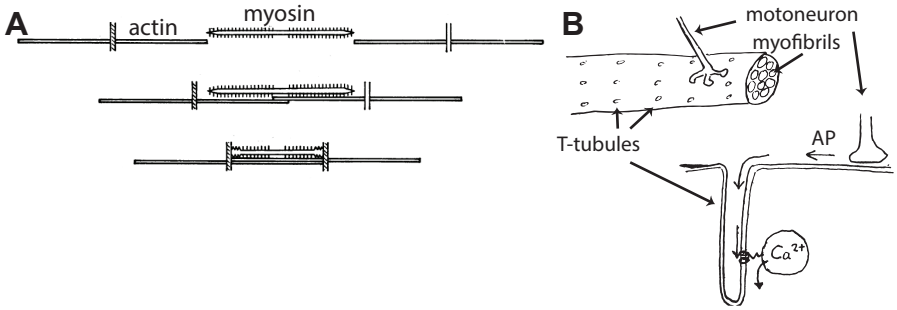

Figure 1.14: Action potentials in muscle. A, Sliding filament theory of muscle contraction: myosin filaments slide on actin, which shortens the fiber (Huxley, 1974). B, Motoneuron contacting a muscle fiber. The AP travels from the motor end plate to the transverse tubule system.

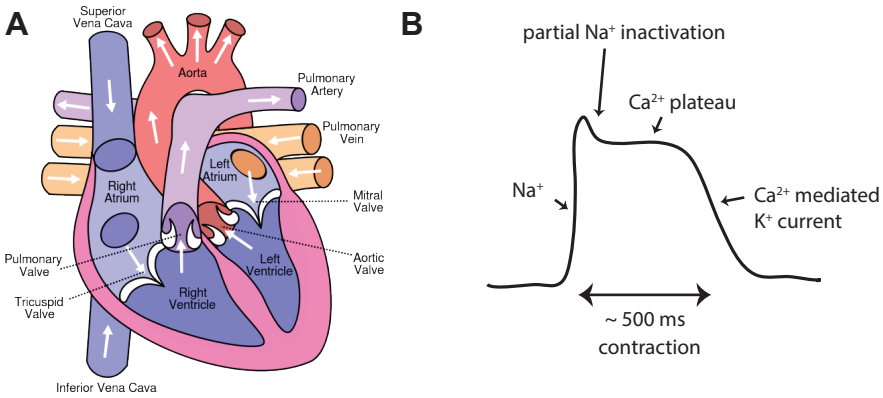

Figure 1.15: Action potentials of the heart. A, Heart anatomy, showing blood flow through the different structures. B, AP of a cardiac myocyte, which triggers muscle contraction.

membrane that run deep into the fiber, making a system of *transverse tubules*. The AP travels into the transverse tubule system, supported by Na channels. There, voltage-gated channels indirectly trigger the release of Ca^{2+}, which in turn triggers the sliding of myosin, resulting in contraction. Fast AP propagation is critical to synchronize the release of calcium in the fiber. Detailed models of AP propagation along muscle fibers have been proposed in the 1970s (Adrian and Peachey, 1973).

Contractions of the heart (Fig. 1.15A) are also triggered by APs generated by muscle fibers, called *cardiac myocytes*. In contrast with APs of skeletal muscle fibers, which are similar to neuron APs, APs produced by myocytes last several hundred ms and consist of three phases: 1) a fast initial Na^+-mediated rise, where K channels close (in contrast with skeletal muscle fibers and neurons); 2) a long plateau due to voltage-gated Ca^{2+} channels, during which Na channels are partially inactivated; 3) repolarization due the inactivation of Ca^{2+} channels and opening of K channels sensitive to intracellular Ca^{2+} concentration (Fig. 1.15B). Proper blood circulation requires the heart to pump blood from the veins into the lungs, then into the arteries. To achieve this, it alternates contractions of muscles wrapping two pairs of chambers, atria and ventricles (Fig. 1.15A). Blood flows in the following sequence: veins, right atrium, right ventricle, lungs, left atrium, left ventricle, arteries. First, the two atria are relaxed, which closes communication with the ventricles, so that blood fills the two atria. Then, atria contract simultaneously, which opens the valves with the ventricles, filling them with blood. Ventricles then contract while atria relax, pushing the blood towards the lungs and the arteries. It is clear that the timing of APs is life-critical: myocytes in atria must fire synchronously, but before myocytes in ventricles. Synchronous firing is achieved by *gap junctions* between fibers, which are contacts between cell membranes where small molecules can flow. The delay between the contraction of atria and ventricles is achieved by propagation delays through small fibers. Finally, rhythmic contractions must be intrinsically generated. Myocytes APs are entrained by a subset of cardiac cells called *pacemaker cells*, which produce APs at a regular rate without external stimulation. This rhythm is also produced by voltage-gated changes in membrane permeability. Detailed models of cardiac APs have been proposed (Luo and Rudy, 1991; ten Tusscher et al., 2004).

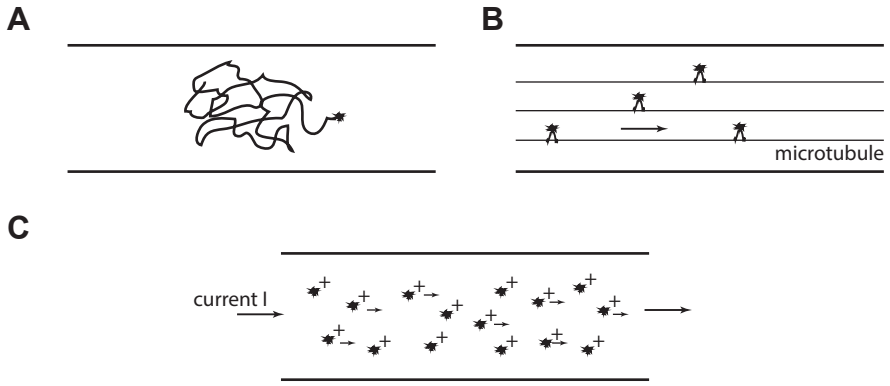

Figure 1.16: Different ways of transporting signals through an axon. A, Diffusion of molecules. B, Active transport along microtubules. C, Electrical transport of charged molecules (ions).

1.3 Why do cells spike?

This brief overview of APs across cells and species allows us to delineate a number of universal features of APs. On the biophysical side, APs are transient increases in membrane potential, by several tens of mV, due to selective changes in membrane permeability to ions. They are triggered by depolarization, whether by an external stimulus (as in Paramecium) or by endogenous events (as pacemaker cells of the heart). When depolarization exceeds a threshold, the AP is produced through a positive feedback loop, independently of the stimulus. This feedback loop is mediated by voltage-gated ionic channels, but not always the same kind (Na^+ in most adult vertebrate neurons, Ca^{2+} in developing neurons, Cl^- in plants). The return to resting potential is often mediated by voltage-gated potassium channels, but sometimes by other types of membrane channels, such as Ca^{2+}-gated K^+ channels in the heart (which open when intracellular Ca^{2+} concentration rises). APs are often all-or-none, but not always. Sometimes, as in Paramecium, the heart, and even vertebrate neurons (Debanne et al., 2013), the amplitude and duration of APs can be graded, in a stimulus-dependent way. In any case, APs are events, i.e., they have an onset time.

On the functional side, action potentials mediate actions. In the squid giant axon, the AP triggers a quick escape reflex. APs of muscle fibers trigger contraction. The Paramecium's AP triggers a reversal in swimming direction. In Noctiluca Scintillans, an AP triggers a flash of light. In the central nervous system, neuron APs produce changes in target neurons (for example, depolarization). Because calcium enters the cell during an AP, APs can also trigger intracellular events. In all these cases, APs trigger an action, a timed event.

Why do cells spike, rather than use some other means of interaction? Let us consider two different viewpoints: communication and action.

1.3.1 Action potentials as messages

Let us first consider the question from a communication viewpoint. How to transmit a message between two points of a cell? Molecules can travel by diffusion under the effect of temperature, following a random walk (Fig. 1.16A). To travel over a distance x, it takes a time of order $t \approx x^2/D$, where D is the diffusion coefficient of the molecule, which is inversely related to the

size of the molecule. For example, K^+ ions have a diffusion coefficient of about 2.10^{-3} mm^2/s in water at 25°C. For Ca^{2+} ions, $D = 0.8.10^{-3}$ mm^2/s. Therefore, it takes about 1 second for Ca^{2+} ions to travel across 100 μm, and time increases quadratically with distance. For larger molecules, travel time can be much longer (about a thousand times for DNA). In summary, diffusion does not allow rapid signaling in a cell, especially over long distances.

Large molecules can be transported actively, along microtubules, which are sorts of railways in the cell (Fig. 1.16B). In contrast with diffusion, active transport is directed and travel time is proportional to distance, but it is not very fast. In axons, the fastest-moving material (vesicles) moves along microtubules at a velocity of about 3 μm/s. Thus, active transport is useful mainly to transport large molecules, but not to rapidly convey signals.

In contrast, electrical interactions act at a distance, and electrically conveying a message between two points A and B does not require movement of matter from A to B. Because charges of the same sign repel each other, injecting a positive current at one end of a conductor (e.g. the axoplasm) means pushing positive charges along the conductor (Fig. 1.16C), which in a cell are mostly K^+ ions [3]. The current is then transmitted almost instantaneously to the other end of the conductor, even though charge carriers move much more slowly. For a conductor where electrons are the charge carriers, current is transmitted nearly at light speed, while electrons themselves move on average at a velocity on the order of millimeters per second.

As a result, in the squid giant axon, the AP propagates along the axon at a velocity of around 25 meters per second. In fact, the net axial movement of ions due to the propagation of an AP is zero, just as alternating current produces no net drift of electrons in a conductor. This is because K^+ ions move in the forward direction at the front of the propagating electrical wave (see Fig. 1.5), i.e., during depolarization, and in the backward direction in the back of the wave (repolarization), producing no net movement. Thus, most movement of ions occurs in the radial direction (through the membrane) rather than in the axial direction (along the axon).

This explains why fast signals, such as those that trigger the escape reflex in the squid, are carried by electricity. However, it does not explain why these electrical signals have to be in the form of impulses, rather than graded signals. For example, touching the caudal end of a Paramecium hyperpolarizes the cell, which in turn increases swimming frequency. The same hyperpolarization is seen all over the cell, which is isopotential, and no AP is produced in this case. But contractions of skeletal muscle fibers are always triggered by APs, rather than graded depolarizations. Why?

Seeing APs as messages, there is a limit to the distance over which an electrical signal can be passively communicated, due to the loss of energy through the axon (as through any conductor). The signal (membrane potential) falls exponentially with distance (a fraction of the signal dissipates on each piece of axon), and therefore it becomes progressively drowned in noise as it travels along the axon (thermal noise and channel noise). This can be overcome by nonlinearly amplifying the signal so that a stable wave (called *soliton* in physics) travels along the axon. In terms of information theory, this occurs at the cost of an important loss of information, since the propagated signal is now discrete, i.e., one bit of information (see Manwani and Koch (1999) for a more detailed information-theoretic analysis). Thus, APs allows a cell to quickly communicate messages over long distances.

1.3.2 Action potentials as actions

Paramecium is essentially isopotential (Eckert and Naitoh, 1970), as are neurons in early development that have short neurites and produce APs before they make any synaptic contact

[3] A positive current also pulls negative ions, but in a cell most of them are large molecules (proteins), which have low mobility; Cl^- ions move at similar speed as K^+ ions, but they are much less abundant.

(Pineda and Ribera, 2007). Thus, there are known cases where APs do not propagate and yet seem to serve some function. In the case of Paramecium, swimming speed is controlled by graded changes in membrane potential, but the cell can also produce APs. For all unicellular organisms, electrical messages do not need to take the form of travelling impulses in order to be communicated across the cell. Therefore, the propagation of messages over long distances does not fully justify the production of APs.

The case of Paramecium illustrates the point that an AP is not only a message to be communicated, it is also a timed decision that triggers an action. A mechanical stimulus on the rostral side, for example, can depolarize the cell, and if it is strong enough (decision), the avoidance behavior is instantaneously triggered. Generally speaking, APs in cells are produced when depolarization exceeds some threshold, that is, it has the nature of a decision, which is a timed event. In the case of Paramecium, a decision has to be taken as to whether the mechanical stimulus should trigger an escape behavior or should be ignored, and since this decision must involve the immediate reversal of ciliary beating distributed all over the membrane, it must be mediated by an electrical event. Before an AP is triggered, the stimulus-induced depolarization is already sensed at all points of the membrane including the cila, and therefore the generation of an AP is not about conveying information. Thus the AP is not so much a message that is conveyed as a trigger for a particular action. In the case of Paramecium, that action is the reversal of ciliary beating direction. In the case of central neurons, the action is generally an effect on target neurons (depolarization, hyperpolarization, or more complex effects). In all cells including developing neurons, the AP also acts on intracellular signalling pathways.

Thus, fast cell-wide coordination requires electrical signaling, which may be graded or event-like. Decisions on an action to be taken require to distinguish between conditions that trigger the action and those that do not, i.e., to set a threshold. By definition, responses near the threshold are unstable, i.e., a small change reverses the decision. In other words, timed cell-wide decisions require an unstable electrical process, which is the AP.

1.4 A few open questions

As outlined above, the biophysical basis of APs is now well known and will be exposed in more detail in the next chapters. What is still lacking is an understanding of the spiking cell as a system, that is, as an organized set of interdependent elements, these elements being the ionic channels, the membrane and its geometry, the intracellular signalling pathways, the genetic machinery, the environment (considered as everything outside the cell). How are these elements interlinked so as to make the cell functional as an AP generation system (function being outlined in section 1.3), all through the life of the cell[4]?

For example, the ability to generate an AP requires a rather precise number of sodium channels in the membrane: the cell is not excitable if there are too few channels, and it fires spontaneously if there are too many channels. The right number depends on the cell's size, which depends on a number of genetic and environmental factors, and increases dramatically over the course of development. How does the cell sets the correct number? The properties of channels also need to be tuned quite precisely: for example, potassium channels should not open too quickly, otherwise they would oppose the sodium current (which is wasteful). When one looks at the cell as a spiking system, it appears that it is highly organized. For example, the spatial distribution of ionic channels is highly heterogeneous, with a "hotspot" near the soma (Fig. 1.10), and different types of ionic channels with spatial gradients of expression and biophysical properties along the few tens of μm of the axonal initial segment (Kole et al., 2008;

[4]which is, for most neurons, the entire life of the organism.

Debanne et al., 2011). High-resolution microscopy has even revealed a robust organization at the submicrometric scale (Leterrier et al., 2015). Even in Paramecium, ionic channels responsible for the AP are not evenly distributed over the membrane but localized in the cilia (Ogura and Takahashi, 1976).

These remarks raise at least two questions. First, what is the significance of this organization, how does it influence AP generation? The theory of excitability was historically developed in large unmyelinated axons, where ionic channels are homogeneously distributed. As shown in Fig. 1.3, the axons of cephalopods are rather peculiar, in that they result from the fusion of hundreds of cells. The theory of excitability developed by Hodgkin, Huxley and colleagues was not about how APs are normally generated in neurons, but rather about the biophysical basis of APs and their propagation along axons. The normal initiation of APs, taking into account the organization of the AP generation system, will be examined in chapter **??**. Second, how does a cell learn to spike, that is, how does it build the complex organization that characterizes the AP generation system of adult neurons? It might be said that a cell learns to spike in much the same way that a child learns to walk: a large part of this process is genetically programmed, but it requires an interaction with the environment and adaptation to the exact configuration of the body. The cell must also maintain its functionality throughout its life, amidst changing external conditions, i.e., it must be "plastic". The development and plasticity of the AP generation system is not very well known. It will be addressed in chapter **??**.

1.5 Epistemological notes

1.5.1 The life of theories

The history of excitability theories illustrates a number of general points about how scientific theories are born, live, die and resurrect (see (Schuetze, 1983) on the history of excitability theories). Some of these points are made in classic epistemological works by Kuhn (1962), Popper (1959), Lakatos (1976) and Feyerabend (2010).

Learning science through textbooks, we tend to see science as a linear process of accumulation of knowledge. But it does not reflect the way science is made. Galvani first correctly attributed the origin of electricity that moved the muscle to the nerve, but this idea was burried by Volta who argued that it came from the dissimilarity of materials (nerve and muscle) and demonstrated it by building the first chemical battery. Although in retrospect this might seem as an error, it was yet quite a rational attitude, since there was much evidence in favor of Volta's theory and no direct evidence in favor of Galvani's idea. It took decades before this view was turned around by Matteucci and Galvani's claim was revived, certainly not without much resistance.

The development of Bernstein's membrane theory is a good illustration of how scientific theories are made. A naive view holds that theories are derived from observations. For example, from the start of an integer sequence 1, 2, 4, 8, we may derive the theory that the sequence is 2^n. This is wrong on several accounts. First, for any finite set of observations, one can produce an infinite number of theories that are consistent with them. This is known as the problem of induction. From this remark, it follows that theories are proposed on other grounds than the observations themselves. In the case of Bernstein, membrane theory was inspired by the work of contemporary scientists on electrochemistry, such as Ostwald and Nernst, and none of that work was on not cell membranes. Thus the theory was not implied by the observations themselves, but rather the observations resonated with chemical theories developed at the time in other contexts.

The second reason why theories are not derived from observations is that observations are actually derived from theories. That is, observations come from experiments that are done within the context of one or several theories. They are not independent of theories because they

are motivated by them, and interpreted in their framework. For example, Bernstein measured the dependence of the resting potential on temperature because electrochemical theory (Nernst equation) predicts a linear dependence, which Bernstein indeed found experimentally. The observations came after and because of the theory. Thus not only theories are not derived from observations, but they are also not made so as to explain observations. The relation between theories and observations is circular rather than causal. This point also explains scientific conservatism, because most data are produced by the dominant theory, which makes it difficult for challenging theories to rise — as the resurrection of Galvani's view against Volta's theory (see Kuhn (1962) for the historical and sociological aspects of science).

The third reason why theories are not derived from observations is that observations are quite often discarded when they do not agree with theory. For example, Bernstein had noticed that the membrane potential becomes positive during the AP, a direct contradiction to his theory that the AP reflects a non-selective increase in membrane permeability. Yet he did not consider his theory falsified, and instead discarded that observation. At first sight, this might seem like an unscientific attitude. But, as pointed out by Lakatos (1976) and Feyerabend (2010), it is a necessary aspect of the scientific process. Any empirical study requires selecting observations that are deemed meaningful, and there are always many potential sources of experimental errors and many observations that have causes that lie outside the realm of the phenomenon of interest. For example, Newton did not consider the fact that heavy objects often fall faster than light objects as a contradiction of his theory. In astrophysics, observations that contradict the laws of gravitation are called planets or dark matter. Reality is complex and no theory can account for all its aspects, and this why no theory claims to account for all observations. In the case of Bernstein, he discarded the contradicting observation of a positive membrane potential as an experimental artifact (recall the measurement devices of the time, Fig. 1.1), and he presumably did so because he had no satisfying alternative framework to explain it. That observation was later confirmed by Hodgkin and Huxley with more reliable measurement techniques (Hodgkin and Huxley, 1939), but Bernstein's view was overturned also because they had a satisfying alternative theory to propose, based on selective changes in membrane permeability, which turned out to be consistent with a number of other experiments.

Karl Popper famously held that science progresses by discarding old theories with *critical experiments*, a view called *falsificationism* (Popper, 1959). This view was motived by his thoughts on the logical structure of theories, which must be falsifiable in order to be considered scientific. Scientific progress then means discriminating between theories by adequately chosen experiments on which theories make different predictions. Superficially, one may see Hodgkin and Huxley's observation of the positive membrane potential during the squid's AP as the critical experiment that discarded Bernstein's theory in favor of their theory. However, as noted above, this was not the first time this observation was made, i.e., the theory had already been falsified. Thus the fallacy of falsificationism is that not only are scientific theories falsifiable, but they are also falsified, all throughout their life. Anomalies in Hodgkin-Huxley theory have also been pointed out and the theory is still disputed today, although marginally (Ling, 1962). The way surprising observations in electrophysiology and other established sciences are interpreted today is generally not by discarding the mainstream theory, but by looking for explanations within that theory (e.g. there is dark matter). This is so because the theory has proven fruitful in a large number of situations. Indeed, electrophysiological theory has been established not only by negative experiments (falsification of alternative theories) but in fact mostly by positive experiments (confirmations), such as the demonstration that APs travel more slowly when the axon is immersed in oil (Hodgkin, 1939).

As outlined above and pointed out by Popper's own students (Lakatos and Feyerabend), the view that science progresses mainly by falsification is neither what happens (Kuhn, 1962) nor

what should happen (Lakatos, 1976; Feyerabend, 2010). On a theoretical ground, one experiment discards an infinite number of theories, and leaves an equally infinite number of theories, all the logical propositions that are consistent with the new observation. Therefore, falsification does not by itself provide a productive way to build scientific theories. Lakatos argued that science progresses rather through a long competition between research programs, which turn out to be more or less productive (Lakatos, 1976).

The history of excitability theories also illustrates the role of tools in science. By allowing new experiments and observations, it is evident that tools play an important role in science. The same naive view that theories are derived from observations would suggest that tools not only are important, but they are the main limiting factor in the production of new scientific theories, since they are the provider of observations. As discussed above, this view is wrong because observations are produced by experiments conceived within the framework of one or several theories. Experiments require tools, and therefore new tools are conceived as means to confirm, infirm or explore aspects of a theory. While new tools may occasionally produce surprising observations that inspire new theories, they do not entirely precede theories.

The history of electrophysiological techniques illustrates this point. For example, Cole built a device to measure the membrane conductance during an action potential, because a key prediction of membrane theory was an increase in conductance (Fig. 1.4A) — which was confirmed. Here is how Hodgkin and Huxley introduce their report of the first use of a microelectrode to record an animal AP intracellularly: *"This potential is generally believed to arise at a membrane which is situated between the axoplasm and the external medium. If this theory is correct, it should be possible to record the action potential between an electrode inside a nerve fibre and the conducting fluid outside it"*. It turned out that the recording also showed a positive membrane potential at the peak of the AP, contradicting Bernstein's theory. Nonetheless, the microelectrode technique was introduced with a particular theory in mind and, most importantly, with ways of interpreting the results. In the same way, the later development of the patch pipette by Neher and Sakmann (1976), which allows recording currents through single ionic channels, was motivated by the theory that membrane currents are produced by the discrete opening of many individual channels, a theory proposed years before and for which there was already indirect evidence based on noise analysis (Katz and Miledi, 1970). The new technique then allowed detailed investigations into the function and structure of channels.

1.5.2 The different kinds of explanation

Why do neurons produce action potentials? If one asks a molecular biologist, an electrophysiologist, a theoretical biologist and an evolutionary biologist, chances are that four different answers will be given. This is what Aristotle called the four causes, or four different types of explanation. All are valid and complementary answers to a why? question, but different communities tend to focus on one type.

The molecular biologist or the geneticist might answer that neurons produce action potentials because they have the genes for sodium channels. SCN8A is one of them, responsible for the expression of sodium channels at the axon initial segment of central neurons of vertebrates. This is the *material cause*. A comparative physiologist, however, would note that many unicellular organisms such as Paramecium can produce action potentials with calcium channels instead of sodium channels, and plants can produce APs using chloride channels. The electrophysiologist might then observe that, independently of the exact protein involved in the phenomenon, APs are produced when the membrane potential exceeds a threshold, above which voltage-gated ionic channels open and let a positive charge enter the cell. This is the *efficient cause*, the causal chain of events that underlie the phenomenon. The theoretical neuroscientist would note,

however, that the presence of voltage-gated ionic channels is not sufficient to create a threshold phenomenon. Those channels must have a certain type of nonlinear properties and be expressed in sufficient amount, relative to the other structural elements of the membrane (e.g. the leak channels), so that a *bifurcation* occurs (a qualitative change in a dynamical system). This is a formal property that may apply to any specific ionic channel, and is called the *formal cause*. Finally, the evolutionary biologist would propose a completely different type of answer, such as those discussed in 1.3, for example: neurons produce APs so as to communicate signals over long distances. This is the *final cause*, that is, a teleological explanation. Many scientists are uneasy with teleological explanations, because they seem to subtend that cells have intentions, when the apparence of goals is only the result of complex molecular mechanisms. But final causes do not need to convey any such implicit meaning. They are also common and useful in physics, where physical laws are described as the minimization or maximization of some quantity (potential energy, entropy). For example, a ball rolling in a bowl will stabilize at the bottom, where potential energy is minimal. One usually does not invoke Newtonian mechanics (efficient cause) to make this prediction but rather the principal of minimal potential energy (final cause). Both principles are of course compatible, and the final cause does not need to imply any intention on behalf of either the ball or some superior being. Final causes are especially important in biology because of evolutionary theory: biological structures have functions in relation with the survival and reproduction of the organism.

This is a theoretical book, which means that special emphasis will be given to formal cause: how the arrangement of structural elements give rise to certain properties. But we will also try as much as possible to explain the articulation between the four different causes, without which no explanation of a biological phenomenon is fully satisfying.

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