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The great Era of English Electrophysiology: from Francis Gotch to Hodgkin and Huxley

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ABSTRACT

Electrophysiology, the science born with the research of Luigi Galvani on "animal electricity" in frog preparations, found its ideal completion in the work of Hodgkin and Huxley on the mechanisms of impulse generation and propagation in the squid axon. The two brilliant English scientists concluded the studies of their great predecessors and teachers, and with their research carried out in the 1950s they laid down a solid foundation for modern electrophysiology.

This paper aims at illustrating, briefly, the history of this fascinating scientific adventure that led to the discovery of some of the most elusive and subtle biological processes present in nature, which has come to the light only through the impressive experimental and logic endeavour of generations of great scientists across the nineteenth and twentieth century.

Key words

History of physiology • Lucas • Adrian • Hodgkin • Nerve conduction • Action potential

German legacy and the cultural context of a "scientific revolution"

The birth of electrochemistry in the second half of the nineteenth century gave an unexpected boost to electrophysiological research. The "membrane theory" elaborated at the beginning of the twentieth century by Julius Bernstein (1839-1917) represented a revolutionary turning-point in the understanding of bioelectric phenomena. As somewhat anticipated by Galvani with his hypothesis of "animal electricity", the plasma membrane of the excitable cells was considered as a barrier separating electricity in a state of disequilibrium. In the view of Bernstein, who relied particularly on the electrochemical theory of Walther Nernst (1864-1941), this separation was the ultimate consequence of the movements of ions present in different concentrations inside and outside the cell, and of the selective permeability of the membrane for these ions. The application of Nernst's law, developed in the context of a thermodynamic approach to electric batteries, represented a first successful instance of a quantitative study of the bioelectric phenomena.

The "*negative Schwankung*", the electric manifestation of signal progression along the nerve, detected by one of Bernstein's teacher, Emile du Bois-Reymond (1818-1896) (and afterwards characterized for its time course and propagation speed by Bernstein himself), was explained by assuming an abrupt and non-specific increase in permeability of the membrane, a functional "breakdown" of its structure. The mechanisms whereby the membrane changed its characteristics remained unclear but the propagation along the fibre seemed to depend importantly on the local flow of current as modelled

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by Ludimar Hermann (1838-1914,) with his "theory of local circuit". The complexity of the mechanism of generation and propagation justified the low speed of transmission experimentally observed by another of Bernstein's teacher, Hermann von Helmholtz (1821-1894), compared to that expected based on analogy with the physical conduction occurring passively along a conductive cable.

The identity between the nerve signal and a negative oscillation seemed to be definitely settled by the perfect correlation between the measurements of conduction speed of the two phenomena carried out respectively by Helmholtz and by Bernstein himself, who succeeded in measuring the speed of propagation of the *negative Schwankung* by using a special instrument he contrived to this purpose, the "differential rheotome". With these achievements it was evident that the animal electricity postulated by Galvani not only existed, but – as already supposed by the doctor of Bologna – was the agent underlying nervous signal transmission.

After Galvani's pioneering research carried out during the eighteenth century and the studies performed in the first half of the following century by Carlo Matteucci (1811-1868), the study of bioelectric phenomena became to a large extent the endeavour of German scientists. However, around the turn of the twentieth century a generation of English physiologists emerged, taking the lead in this field, and inaugurating a period that can be defined as "the great era of English electrophysiology ": Cambridge neuroscientists began their research on nervous propagation and, in particular, they pointed to the non-decremental character of nervous conduction, a property largely differing from the passive conduction of electricity in an ordinary physical cable.

Lucas and Adrian: an elegant investigation about nervous propagation

In 1850, in relation with his successful measurement of the nervous conduction speed, Helmholtz considered two different models which could in principle account for the propagation of the nervous signals. He wrote: "The conduction in the nerve would belong to the series of these molecular effects that are transmitted in ponderable bodies, among which effects include for example the conduction of sound in air and in elastic materials or progressive firing of a tube filled with explosive mixture" (Helmholtz, 1850: 330).

The metaphor assimilating nerve conduction to the spatial progression of an explosive phenomenon was first hinted by Helmholtz, and re-emerged with the gunpowder tracking image used by two great Cambridge physiologists in the second decade of the twentieth century. It is very interesting to read a significant part of what they wrote in a book – initiated by Keith Lucas (1876-1916) but edited and brought to completion, after Lucas sudden death in an air accident, by his pupil Edgard Douglas Adrian (1889-1977) –, entitled *The Conduction of the Nervous Impulse* and published in 1917: "A disturbance, such as the nervous impulse, which progresses in space must derive the energy of its progression from some source; and we can divide such changes as we know into two main classes according to the source from which their energy is derived. One class will consist of those changes which are dependent on the energy supplied to them at their start. An example of this kind is a sound wave or any strain in an elastic medium which depends for its progression on the energy of the blow by which it was initiated. A sound wave will soon lose its initial energy if the medium in which it progresses is imperfectly elastic, because the medium will be heated in its deformation. Suppose a sound wave travelling through air and then encountering a tract of treacle. In its passage through the treacle it will lose its energy more rapidly than in its passage through the air, but on emerging into the air again it will have suffered permanent loss, and will not recover the energy which it had before it entered the treacle. A second class of progressive disturbance is one which depends for its progression on the energy supplied locally by the disturbance itself. An example of this type is the firing of a train of gunpowder, where the liberation of energy by the chemical change of firing at one point raises the temperature sufficiently to cause the same change at the next point. Suppose that the gunpowder is damp in part of the train; in this part the heat liberated will be partly used in evaporating water, and the temperature rise will be less, so that the progress of chemical change may even be interrupted; but if the firing does just succeed in passing the damp part, the progress of the change in the

dry part beyond will be just the same as though the whole train had been dry" (Lucas and Adrian, 1917: 23-24).

The idea that nervous signals could propagate without attenuation arose, by generalization, from some stimulus-response relationships observed in muscles fibres. It was common knowledge for already several decades that, while the heart exhibits an all-or-none type of response to electric stimuli of increasing intensity, skeletal muscles contract with a progressive increase of strength as the stimulus amplitude is increased. In 1902, another English physiologist, Francis Gotch (1853-1913) thought that even muscle fibres responded, in reality, in a "binary", all-or-none type to the electric stimulation and that, the apparently continuous increase of strength was caused by the recruitment of new fibres (the great number of the recruited fibres preventing the manifestation of the discontinuous character of the contraction of any individual fibre; Gotch, 1902). In 1905, Keith Lucas reduced surgically a frog muscle to a few fibres and demonstrated that the increase of contraction strength is really basically discontinuous (Lucas, 1905). He obtained discrete increments which were equal (or less) than the number of the remaining fibres in the "reduced" frog muscle. In 1909 Lucas came to the same conclusion in stimulating a nerve reduced to a few fibres (Lucas, 1909). Only in 1926, by using a capillary electrometer, Adrian succeeded in obtaining an instrumental measuring of the single event underlying the propagated disturbance, finally directly revealing its all-or-none character and, thus, confirming the existence of a self-regenerative process at the base of nervous propagation (Adrian, 1926).

Between the experiments of 1909 and the recordings of 1926, however, there was a phase of intense experimentation on the propagation of the nervous signals, with its supposed non-decremental character supported by the results of some research and questioned by the results of other studies. A protagonist of this research was the same Adrian, initially together with his teacher Lucas, and then, alone. Adrian carried out a series of experiments on nerve block, utilizing muscle contraction as a monitor of nerve conduction (Adrian 1912, 1914). He set up experiments to interfere with the mechanism responsible for self-regeneration and propagation of the supposed all-or-none phenomenon of disturbance, by appropriately narcotizing segments of the nerve on various lengths, causing a simple passive conduction with progressive decrease of the signal amplitude.

Adrian measured the time necessary to reach the block of nervous conduction after application of the narcotic treatment ("extinction time"). He supposed that a linear relationship would exist between the length of the treated fibres and the decrement of signals amplitude along narcotized tracts; and, moreover, the spatial slope of the decrement would depend on the intensity and duration of narcotic treatments. A first result was that a shorter extinction time was required with an increase of the length of narcotized tract. Adrian explained this result by considering that the same "slope" of decrement in a longer tract would cause in a gradual fashion the achievement of the blocking threshold in a shorter time. The crucial experiment, published in 1912, used two narcotized segments of equal length, separated by a non-treated segment of adequate length. Since both segments had been treated the same way, it was assumed that the same decrement would be produced in each of them. If, moreover, there were no signal regeneration in the middle untreated segment, the total decrement should be equal to the sum of the two decrements taken separately (and similar to that of a long segment of twice this length). A corresponding event should occur with the extinction time. The result did not confirm this expectation. With the narcotization of two short segments separated by an untreated segment, the extinction time was similar to that of the treatment applied to a single short segment. This result was accounted for by assuming that the signal conduction implied a regenerative mechanism for its propagation along the nerve. In the case of a treatment incapable of producing a complete blockade by the application to a short segment, the signal would regenerate and recover the signal amplitude in the untreated segment before invading the next treated segment (Fig. 1).

On the basis of many studies of the nerve signal generation and propagation, carried out on a variety of nerve fibres, Adrian came to formulate the first hypothesis that frequency modulation coding is a universal mechanism underlying signals propagation in excitable fibres.

Regarding Adrian's study of nerve narcotization, it must be said that, despite the validity of the general conclusions they promoted, the interpretation of the

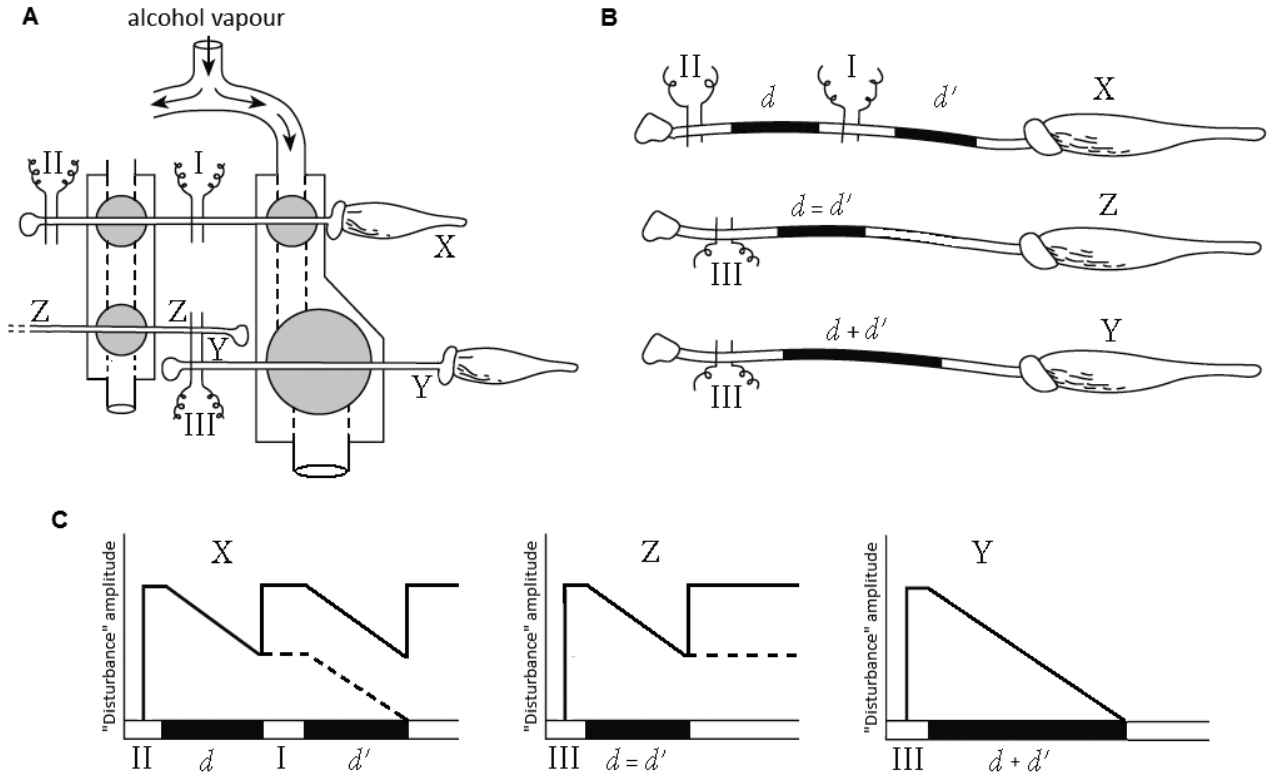

Fig. 1. - Adrian's experiment of nervous block by alcohol vapour. A) Experimental system used to check simultaneously the effect of alcohol vapour on three different preparations of nerve-muscle of frog (X, Y and Z). B) Schematization of different narcotized tracts (in black) and stimulating electrodes (I, II, III) in three different situations, respectively the case of two equal length narcotized segments, only one treated segment of identical length and finally, the case of only one segment of double length. C) Diagrams of different possible results in the three different conditions: solid line shows the case of non-decremental transmission, while dashed line shows the decremental type of propagation: detailed explanations are found in the text (from Adrian, 1912, widely modified by Daniele D. Marasco, 2010). It is very interesting to note that Adrian's experimental results were the outcome of fortuitous experimental choices, depending mainly on the specific length of the treated segments. Nevertheless, Adrian's conclusions about the non-decremental propagation of the nervous impulse were correct. Gen'ichi Kato, who reached the same conclusion as Adrian about self-regeneration of the nervous signal, formulated assumptions very different from those of Adrian. This was because of his different experimental preparation (with particular reference to the geometric choices).

blockade mechanism was more complex than was assumed by the English physiologist.

We know today that the action potential travelling along a narcotized tract has a more complex behaviour compared to Adrian's assumptions. When it encounters the treated area, where maximum sodium conductance is reduced, it initially undergoes some changes in its size, shape and conduction velocity. Propagation is thus altered, but still persists in its self-regenerative character. Proceeding with the intensity of narcotization, it will come to a point where self-regeneration will be simply impossible and the nervous signal will turn abruptly to a purely passive propagation that will extinguish entirely, because of the high longitudinal resistance of a nervous fibre within a short distance depending strongly on the fibre diameter (Fig. 2). A computer simulation of this behaviour has been realized by the author and it is available at this web link: http://youtu.be/PvspclYso6c. In other words, despite the fundamental historical importance in pinpointing the regenerative character of nervous signal propagation, Adrian's experiments were based on assumptions not supported by subsequent work, such as the exclusively passive propagation of the signal in the segment of nerve undergoing the blockade. During

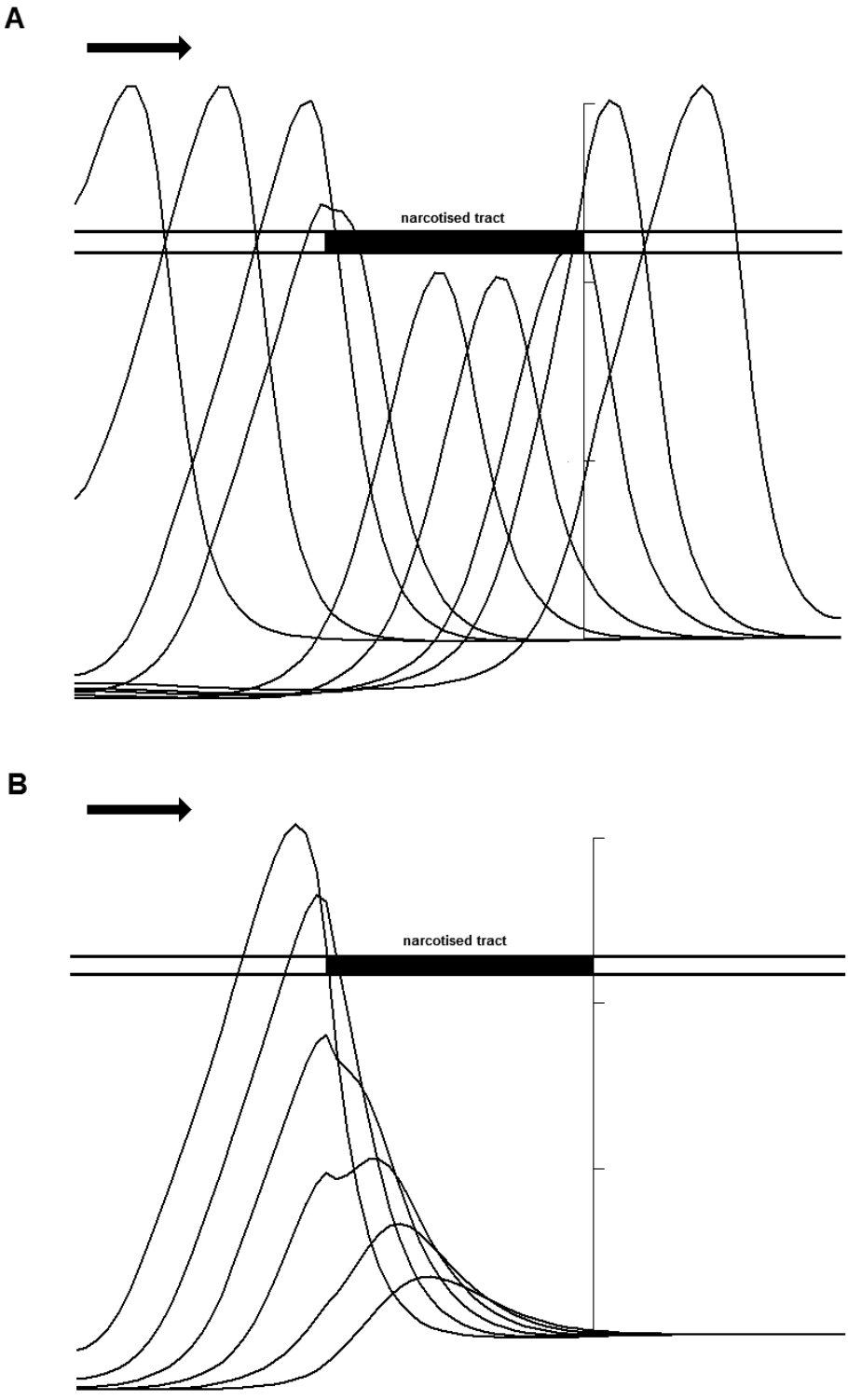

Fig. 2. - Images from two simulations realized by the author based on the NEURON software (Hines and Carnevale, 2006). A) A two-centimetre segment (in black) of a non-myelinated nerve is "lightly" narcotised (small reduction of sodium maximum conductance): the nervous impulse changes its shape, conduction speed and amplitude, but maintains a self-regenerative character and recovers its original form travelling beyond the treated segment. B) With a stronger narcotization the nervous impulse falls quickly: it turns abruptly towards an electrical passive response which extinguishes itself totally in a few millimetres (the different traces shown are not taken at regular intervals of time).

Adrian's time, a controversy arose on the interpretation of nerve conduction blocking experiments with the Japanese physiologist Gen'ichi Kato (1890-1979) (Piccolino, 2003, 2008).

Passive currents are responsible for the spatial propagation of the nervous impulses and, also, they play an important role in nervous communication by being responsible for the space-time integration of inputs which come from the other nervous cells (these currents even represent the only kind of communication in some small nervous cells). The understanding of the mechanisms underlying the generation and conduction of the nervous impulse was achieved by Alan Hodgkin who investigated and correctly interpreted the temporal and spatial relationships between these passive phenomena and connected them, finally, with the explosive event we call *action potential*.

Hodgkin takes Adrian's paths

At the beginning of his research career, Adrian's pupil Alan L. Hodgkin (1914-1998) began an intense and fruitful experimentation aimed to investigate the elusive mechanisms underlying the nervous impulse. The brilliant intuition of this young English scientist led him to focus initially on the study of those little decremental phenomena which precede the firing of action potential. He began with the same instrumental means of his teacher and with a remarkable experimental continuity with his teacher work, although he started working on the subject some twenty years later.

In 1937 Hodgkin narcotized a nervous segment, stimulated simultaneously either the upstream or the downstream tract and found an increase of excitability of the downstream segment. He also measured, with extracellular electrodes, the small electrical responses from the downstream tract, where they appeared as passively propagated phenomena, having the same polarity of the regenerative responses detectable from the upstream segment (Hodgkin, 1937).

In 1938 Hodgkin discovered that passive responses, similar to those found downstream of the narcotized segment, could also be recorded near the application site of a subthreshold stimulus. Proceeding in this direction, he began to vary the intensity and polarity of the stimulus, recording the responses with surface electrodes placed near the stimulating electrode. He found that when the polarity of the current stimulus was such as to increase the pre-existing separation of charges (hyperpolarizing stimuli), the passive responses were progressively larger with increasing intensity of stimulation. On the contrary, in the case of depolarizing stimuli of increasing amplitude, there were three different, consecutive, responses partially merging one in the others: a passive response of progressively increasing amplitude; a passive response with a marked depolarization tail; and a sudden onset of an impulsive event of great (and uniform) amplitude. These experiments seemed to confirm the role of the weak local currents supposed by Hermann in the propagation of the action potential (Hodgkin, 1938).

In 1939 Hodgkin demonstrated that the conduction velocity of the nervous impulse could be varied by reducing the volume of extracellular fluid and replacing it with other fluids of different electrical resistance or, even, by replacing it with other conductive means (according to various experimental configurations). These results confirmed incontrovertibly that an electric process is a necessary link in the progression of the signal along the nerve fibre! (Hodgkin, 1939).

During the same year, working with another great English scientist, Andrew F. Huxley (born 1917), Hodgkin carried out intracellular recordings from the giant squid axon (a little less than one millimetre of diameter; Hodgkin and Huxley, 1939). These recordings showed not only the exact time course of action potential, but also the existence of an internal-negative resting potential, as correctly supposed by Bernstein. In contrast, however, to Bernstein's hypothesis based on the assumption of an indiscriminate increase of membrane permeability to all ions during the excitation, the study of Hodgkin and Huxley pointed to a different mechanism for the action potential. It was found that during the excitation, the membrane potential did not simply reached the zero level (as should according to Bernstein) but inversed polarity, the internal side of the membrane becoming positive with respect to the external side. It is curious to notice that although the "overshoot" of membrane potential during nerve excitation became a fundamental element of further progress in the field, it had already been observed by Bernstein himself. The German scien-

tist had, however, failed to grasp the importance of his observation.

In 1949, working with the English physiologist of German origin Bernard Katz (1911-2003) Hodgkin showed the reduction of action potential amplitude by varying the extracellular sodium concentration, focusing attention on this ion ignored until then (Hodgkin and Katz, 1949). These experiments pointed to two important characteristics of the nerve excitation process which were not in line with Bernstein's hypothesis: a) the possibility of a selective (i.e. non-indiscriminate) variation of membrane conductance during excitation, and b) the central role of sodium ions as main responsible for the impulsive depolarization of the nervous signal.

To understand the crucial experiments that led Hodgkin and Huxley modelling nervous impulse behaviour, it might be useful to mention the electrophysiological technique developed in 1949 by Kenneth Stewart Cole (1900-1994) and George Marmont (1914-1983), two American electrophysiologists. As we know the action potential is an explosive event, which tends to occult the smaller electrical phenomena which are essential for the genesis and propagation of nervous signals in excitable cells. The main ionic conductances involved in action potential firing are a time-dependent function of the electric field that permeates the membrane (and, thus, of the membrane voltage). As the conductance for an ionic species changes, there is a change of the transmembrane ionic flux of the specific ions, potentially resulting in a change of the membrane potential similar to that which has produced it. In order to study the ionic conductance at a given potential, it is necessary to keep the potential constant, by counteracting the possible ionic fluxes, and thus preventing the potentially explosive character of the membrane events promoted by electric stimuli.

The voltage clamp is a technique that, by using a relatively sophisticated electronic apparatus, based on a feed-back process, "corrects" moment by moment the changes in membrane potential, measuring at the same time the amount of current necessary to keep membrane voltage at the desired value, despite any regenerative process. This technique, which was initially applied to the giant axon of the squid by utilizing a longitudinal electrode inserted into the fibre, also ensured a kind of "space clamp", making virtually isopotential the entire fibre (thus simplifying the study of the phenomenon; Marmont, 1949).

Hodgkin and Huxley's model

Hodgkin and Huxley carried out their memorable voltage clamp experiments on the giant squid axon. In order to model the behaviour of the biological components of the membrane (phospholipidic bilayer, ionic permeabilities, active transport systems), they developed an equivalent electrical circuit incorporating various elements (electric batteries, fixed or variable conductances, capacitances: Hodgkin and Huxley, 1952a, 1952b, 1952c, 1952d). By relying on the experimental data obtained in voltage clamp experiments, and applying the principles and laws of electric circuits, it was possible to adopt the mathematical formulae already known and used in other fields to achieve a quantitative description of bioelectric phenomena very close to experimental reality.

The first equation of Hodgkin-Huxley's (H-H) model is based on the application of Kirchhoff's laws and represents the balance of currents (either ionic or capacitive currents) that at any given time flow trough the membrane. In absence of extrinsic currents (or electrical ionic diffusion from neighbouring sites, as it occurs normally during action potential propagation), the total current must be zero. Concerning the time and voltage variability of sodium and potassium conductances, Hodgkin and Huxley supposed the existence of molecular gates (today known as ionic channels) made up of one or more "particles" sensitive to the electric membrane potential which could take one of two possible states: open or closed.

The transition between the two states is a Markovian stochastic process with a probability depending on the membrane electric field. It requires a finite time to occur, according to precise kinetic constants: every conductance, at every given time, is equal to the respective maximum conductance multiplied by the fraction of relative open channels (in other words: the probability that the "particles" constituting them are simultaneously open).

To "resolve" the model and obtain the form of action potential, it was needed to carry out a long series of voltage clamp experiments and data analysis in order to find the relationship between membrane voltage and kinetic constants, and finally to "sketch" the time-course of membrane and propagated action potential.

The mathematical complexity of the H-H's model reflects the complexity of the physiological mechanisms at the basis of the generation and propagation of the nervous impulse. But the model results are extremely precise and still represent the cornerstone of modern cellular electrophysiology. The model points to the existence of a positive feedback mechanism (today known as "Hodgkin cycle"): sodium ionic channels open progressively with the achievement of a specific threshold of membrane voltage in determining a further (and faster) depolarization which follows the opening of other channels, and so on. The process is, however, self-limited because of a spontaneous channels inactivation and is followed by a status of unresponsiveness until a new repolarisation.

Also potassium conductance increases with depolarization, but without undergoing (at least in the original model) any inactivation process. The flux of potassium ions contrasts with the depolarizing effects of sodium current (which prevails only during earlier phases of the action potential), leading the membrane voltage back to its resting value (Fig. 3). Positive charges entered into the cell also tend to spread around, thus modifying the adjacent plasma membrane and permitting the propagation of nervous impulse.

Conclusion

It might be interesting to conclude with a non-trivial question: "Why did Nature contrive a process of propagation so articulated, slow and metabolically expensive?".

The answer to this question lies in the physical characteristics of nerve fibres: their nature, length and thinness render impossible the passive electrical transmission on "large" distances. As put forward in a clear-cut image by Alan Hodgkin, the longitudinal resistance of a long and thin nerve fibre can be as high as that of a thick metallic cable several times the distance from the Earth to the planet Saturn (Hodgkin, 1964). Electrical transmission systems based on cables (as in telegraph lines) have along their course some relay stations, where the signal is received, amplified and transmitted to the next station. In a similar way, nervous fibres can amplify the signal which tends to be degraded in its propagation along them.

Myelinated fibres in particular act similarly as cables: the Ranvier's nodes regularly distributed along their length function as receiving and transmitting stations: they receive the passive currents coming from the preceding node and, through a complex machinery (based on local energy and volt-

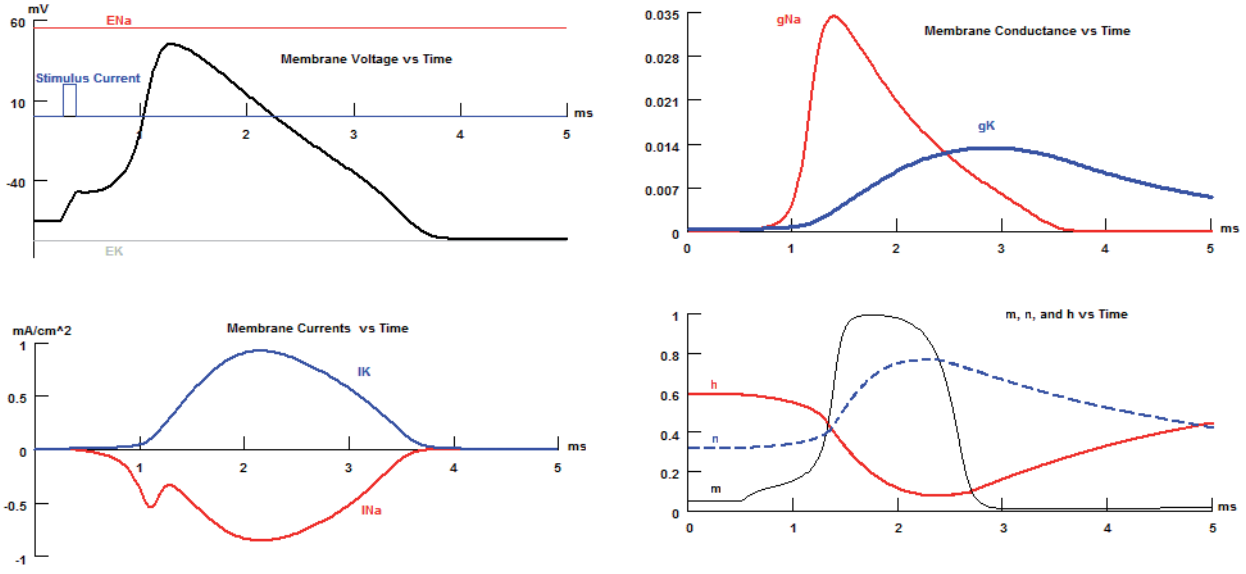

Fig. 3. - Simulation based using the NEURON software (Hines and Carnevale, 2006), realized by the author: time-course of main electrophysiological quantity during AP firing in an isopotential patch, according to H-H's model predictions.

age-gated channels), they trigger Hodgkin's cycle. A new full amplitude impulse is thus generated and travels towards the next node.

The conclusion of the long path of experimental research started in 1780 with Galvani's experiments on frogs and ideally concluded in 1952 with Hodgkin-Huxley experiments in giant squid axon is that nerve signals are electric but they are basically different from the flow of an electric current along a conductive cable. And this happens for well-defined physical reasons.

Said otherwise, Nature is subtle but not (unnecessarily)… malicious!

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