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# The 60th anniversary of the Hodgkin-Huxley model:

# a critical assessment from a historical and modeller's viewpoint

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# Preface

Mathematics is a powerful creation of the human mind. Like English, Dutch, Italian, German, mathematics is a language. It is the language that is universally recognized in the scientific community as the most adequate to describe, predict, and eventually explain the phenomena we observe. There is one very fundamental reason for this: mathematics is, at the same time, flexible and precise as no other known language is. These two properties (flexibility and precision) make mathematics an exceptionally powerful tool. Looking back to the history of Science, we may find however cases in which the mathematical appearance of certain theories masked underlying unjustified hypotheses and distracted from critical thinking. In this thesis we provide a concrete example of one of these cases: the Hodgkin-Huxley model, nowadays the most popular mathematical model in the neurosciences. It will be shown here that although this model has a remarkable descriptive power, it has some major flaws from the explanatory perspective of the phenomena it describes.

My interest in critically studying the Hodgkin-Huxley model arose from numerous discussions with Prof. Dr. Konrad Kaufmann, during my staying at the Max Planck Institute for Dynamics and Self-Organization in Göttingen. I decided then to choose this specific topic for my thesis for two reasons: (a) I have been studying neuroscience since more than three years now, actively working in the field at the MPI since more or less two; and (b) it happened that this year the 60th anniversary of the publication by Hodgkin and Huxley of their theory has been celebrated with a World conference on computational neuroscience, where it happened that no substantial criticism to the model was advanced. It goes without saying that, except from extremely rare exceptions, the same celebrative attitude towards the model is observed through all the neuroscientific community, while the diverse experimental evidences against some of its most important hypotheses are still ignored. It appeared then to me that a comprehensive, critical treatment of the mathematical theory advanced by the two Nobel-awarded physiologists would have been important, both for reconstructing the historical development and derivation of the model, and for providing a critical assessment of the experimental evidence used to support the claim of the existence of an explanatory power of the model.

The results of my efforts - necessarily highly interdisciplinary, at the interface

between mathematics, biophysics and electrophysiology - are reported here in this thesis for obtaining the Master degree in Applied mathematics. The mathematical content mainly focuses on the techniques used in deriving the models, in particular the one from Hodgkin and Huxley in its static and propagating form, and the identification of the relationships among them. A detailed analysis of the behaviour of possible solutions of the Hodgkin-Huxley model was beyond the scope of this thesis (and mathematically still a topic of advanced research). More importantly, it seems reasonable to say that one should first establish (or disprove) the value of the Hodgkin-Huxley model as an explanatory model with proper hypotheses supported by experimental observations, before starting such an analysis. For similar reasons, we did not discuss in depth simplifications of the Hodgkin-Huxley model like for example the FitzHugh-Nagumo model, which is only briefly mentioned here. These have even less explanatory power than the detailed model they somehow approximate.

I hope to have managed in such an intent to provide a valuable reading.

Göttingen, April 2013

Acknowledgements

First of all I would like to thank my thesis supervisors Prof Dr Marc Timme and Dr Sander Hille for trusting in my work and giving me the possibility to pursue my way in Science. Without their open minds this work would have never been possible. I am deeply grateful also to the librarians of the Otto Hahn Library (Max Planck Institute for Biophysical Chemistry) for their kindness and valuable help in providing me hardly-accessible articles and books. I can't imagine my work if their help would be missing. I thank Prof Dr Konrad Kaufmann for the seeds of doubt, and Dr Ahmed El Hady for numerous discussions on experimental neurophysiology; I thank Marta, for trusting and feeding our Future. I dedicate this thesis to my parents, as a filtered drop of a whole thing they still can't see, but always sustained.

# Contents

CHAPTER I

# Introduction

In the history of the biological sciences, there exists no mathematical model that has been welcomed with such a broad consensus as the Hodgkin-Huxley model. Since its publication in 1952, the theory developed by the two physiologists from Cambridge University laid the foundations for the interpretation and planification of experiments, for the understanding of diseases and illness conditions as well as for the design of new drugs. Nobel prizes have been awarded for having conceived techniques which could be used to collect data interpretable as if in support of the theory (eg. Neher and Sakmann, Nobel Prizes for Medicine or Physiology 1991 "for their discoveries concerning the function of single ion channels in cells") or for having elucidated the fine structure of macromolecules especially relevant within the framework of the model (eg. MacKinnon, Nobel Prize for Chemistry 2003 "for structural and mechanistic studies of ion channels"). Nowadays in every university, every neuroscience course includes at least one lecture dedicated to the mathematical interpretation of nerve excitation given by Hodgkin and Huxley.

## 1.1 Objectives of the thesis

This year, the 60th year since the publication of the model of the action potential, the Organization for Computational Neurosciences celebrated the recurrency by helding a congress at the Alma Mater of the two scientists, namely the Trinity College in Cambridge. On the webpage of the event one could read:

*"This publication* [Hodgkin and Huxley 1952] *and the mathematical model it describes is at the core of our modern understanding of how the action potential is generated, and has had profound effects on many fields of biological science in particular on computational studies of neural function"*

The Journal of Physiology - the journal where the model was originally published as well as one of the most influent journals in physiology since more than one hundred years ago - dedicated the issue of June of the current year to the epoch-making achievements and legacy of the Hodgkin-Huxley model. In the articles published, there appear sentences such as:

*"It* [the HH model] *remains one of the best examples of how phenomenological description with mathematical modelling can reveal mechanisms long before they can directly be observed"* (Schwiening 2012)

or

*"The modern era of research on electrical signalling in nerve, muscle and other excitable cells began in 1952 with a series of four seminal papers by Hodgkin and Huxley on analysis of the action potential of the squid giant axon using the voltage clamp technique"* (Catterall 2012)

and even

*"Looking forward, we expect that the Hodgkin-Huxley contribution will continue to propel biomedical research, in areas as diverse as muscle physiology and pharmacology, autonomic physiology, neuroscience disease patophysiology and even clinical medicine"* (Vandenberg and Waxman 2012)

In this work we show that the model developed by Hodgkin and Huxley cannot be considered valid in its full generality. This not only because it has obvious discrepancies with what could in principle be defined fine details such as for example with some specific neuronal behaviours, but because the very fundamental aspects of the theory do not conform with experimental evidence.

The thesis is structured as follows:

After an introductory section on the basic concepts of neuroscience (Section 1.2), an in-depth analysis will be provided of the major scientific influences of the two physiologists (Chapter II). The purely qualitative as well as the quantitative ideas (models) that led to the development of the Hodgkin-Huxley theory will be analyzed.

The third chapter is dedicated to the model as originally conceived in 1952. There the derivation of the equations for both the static membrane voltage variation and the propagated action potential will be treated in detail.

Chapter IV deals with the critics to the model. Here I will focus on the most fundamental of the assumptions made by Hodgkin and Huxley: the hypothesis that the inward flow of sodium ions is responsible for the generation of nerve excitation. The inconsistency of such a claim will be shown first on the basis of the experimental evidence, then on the theoretical level.

A final section in which the curr ent misunderstanding of the predictive and descriptive power of the model will discussed, concludes the work (Chapter V). Possible future directions will be shortly outlined.

## 1.2 Fundamentals of single cell neurophysiology

This section is based on (Kandel et al. 2000, Purves et al. 2008, Hille 2001, Heimburg 2007).

Neurons are the cells of the nervous system. From a morphological perspective, most of them share a characteristic shape in which a dendritic tree is connected to a soma, in turn connected to an axon and its terminals (Figure 1.1). The peculiarity that made neurons become so popular in physiology is their capability to communicate over long distances via the generation, propagation, and transmission of electrically measurable states of excitation. But what is in fact neuronal excitation? Or, better, what do we mean nowadays with this term?

Let's focus on one single neuron. During its life, this will receive several inputs at its synapses located at the end of the dendritic tree. These inputs, normally mediated by chemical compounds called neurotransmitters, if strong enough will perturb the neuron to the point that its constituent structures, the membrane in particular, will be destabilized. Such a perturbation propagates along the dendrites, reaches the soma and converges into the axon where an even greater alteration occurs as a consequence of the superposition of multiple inputs coming from different dendrites. Along the axon, which can be thought of roughly as a long cable, the local alteration spreads until it reaches the terminals, where the perturbed synapse will finally release its own neurotransmitters towards the neighboring neuron, in this way transmitting the excitation.

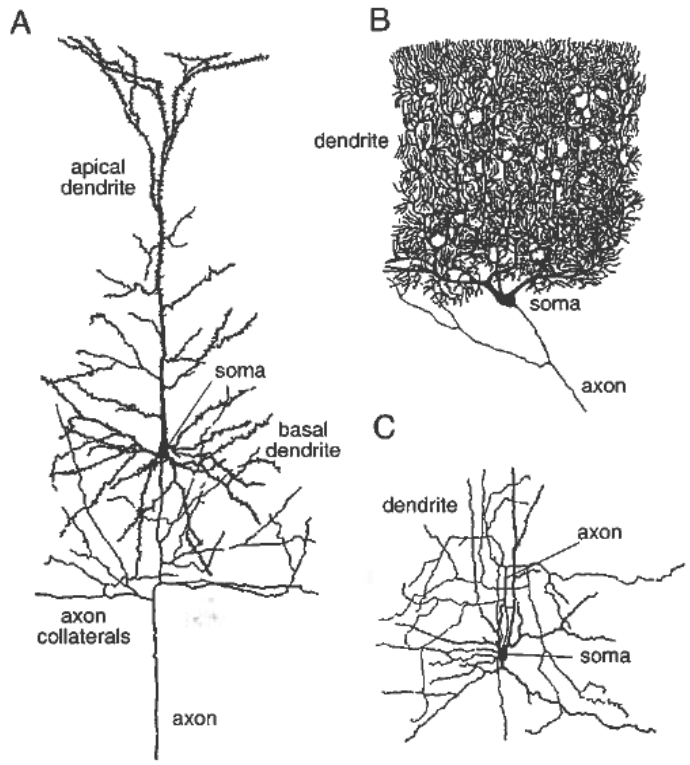


Figure 1.1. Examples of neurons: (a) a cortical piramidal neuron of the cerebral cortex; (b) a Purkinje cell of the cerebellum; (c) a stellate cell of the cerebral cortex. Reproduced from (Dyan and Abbott 2001).

Nowadays we know that the perturbations of the neuronal structures manifest themselves in several (unseparable) ways, as for example electrical, temperature, and pressure signals. Due to historical reasons, however, the first of these signs has received far greater attention than the others; this in turn has led to the widely spread misinterpretation of neuronal excitation as a purely electrical phenomenon. Although this is clearly not the case, being the classical interpretation of nerve excitation the focus of this thesis, electricity alone will be treated in the following chapters. In the next few lines I will thus just briefly introduce the concept of membrane potential and mention the techniques commonly used to measure it.

Neuronal membranes are bidimensional structures mainly composed of lipids and proteins. They separate the intracellular space from the extracellular one and are selectively permeable with respect to ions; in particular, membranes are largely impermable to the macroscopic negatively charged ions that constitute the cellular skeleton (intracellular proteins), while being permeable to the small ions that are dissolved in solution such as sodium or potassium. The concept of semipermeability has long been extended to the in fact never properly tested claim of the presence of specific pathways across the neuron for small ions too. According to this interpretation, there should exist protein-channels embedded in the membrane which are capable of allowing the passive flow of certain paricles and not of others (for example potassium but not sodium). In this way, only the ions whose correspondent channels are open are free to equilibrate across the membrane according to chemiosmosis, the others being constrained at one or the other side of the membrane.

In the resting, non excited state, only potassium channels are thought to be open. As a consequence, potassium ions but not the others will equilibrate. Consider the simplified example in Figure 1.2: initially the membrane is taken to be impermeable, and at both of its sides electroneutrality is assumed to hold, the amount of negatively charged ions A and of positively charged ions K being the same within each compartment (right and left). If now the membrane is rendered permeable only to K, K will start to diffuse until an equilibrium will be reached between the osmotic force due to the concentration gradient and the electric force due to the generated unbalance of charges at opposite sides. In the new conditions, a potential difference across the membrane will be measured with the left side being more negative than the right one. The magnitude of such potential difference can be calculated with good approximation using Nernst's equation

$$E_K = \frac{RT}{zF} \log \frac{[K]_l}{[K]_r}.$$

where $[K]_l$ and $[K]_r$ are the concentrations of the ion species $K$ respectively at left and right of the barrier, $R$ is the ideal gas constant (8.314 J/mol K), $T$ the temperature in Kelvin, and $F$ the Faraday constant (96485 C/mol). In pretty much the same way, the membrane potential of neurons at resting conditions is normally estimated using the equation above for potassium ions. Being potassium normally highly concentrated inside neurons and rather diluted outside, the membrane potential is normally expected to be negative. This prediction

has received experimental confirmation, the usual values of the potential being around -50 mV.

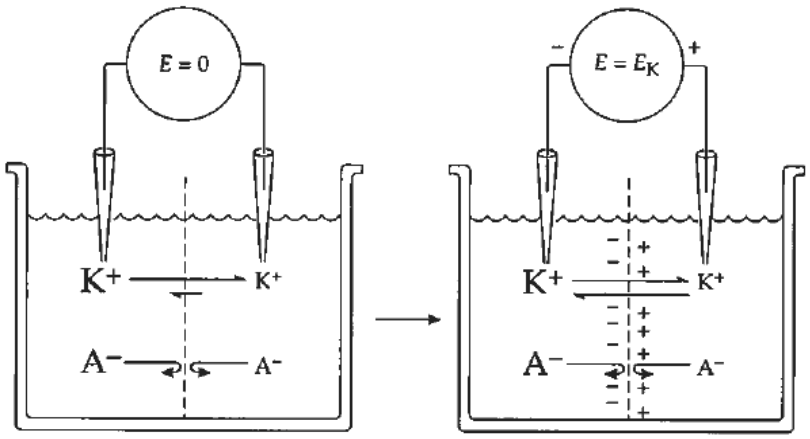


Figure 1.2: Potential across a membrane. The membrane is first considered impermeable to all ions dissolved and no potential difference is recorded (left); when the membrane becomes selectively permeable to K, the membrane potential eventually reaches the Nernst potential for such cation (Right). Reproduced from (Hille 2001).

During excitation, since the classic work of Hodgkin, Huxley and Katz in the late 40s, the membrane is assumed to become selectively permeable to sodium and poorly or non-permeable to potassium. In other words, during excitation sodium channels are expected to open and potassium channels to close. Given that at resting conditions the concentrations of sodium are roughly the opposite of the ones of potassium - in the squid axon under physiological conditions, for example, $[K]_{inside} = 400\text{mM}$, $[K]_{out} = 20\text{mM}$, while $[Na]_{inside} = 50\text{mM}$ and $[Na]_{out} = 440\text{mM}$ - a (large) reverse in potential should (and in fact does) occur when the neuron is active. Sodium channels would then start to close, potassium channels to open, thus causing a decrease in the membrane potential back to the resting values [1]. This wave in the transmembrane voltage is usually what neuroscientists refer to with the term "action potential".

[1] To be more precise, the original resting membrane potential is restored also thanks to "active transporters", i.e. proteins which actively pump sodium outside the neuron. As this is at the moment not necessary and at the same time would add a certain degree of complexity

Experimenally, action potentials can be both induced and recorded since long time with the help of electrodes. In single giant neurons in particular, stimulation is commonly achieved by placing anode and cathode in contact with the external surface of the cellular membrane and injecting current. Under these conditions, physiologists distinguish between two cases: cathode and anode excitation (depending on close to which electrode the neuronal perturbation originates). No qualitative differences are normally observed among cathode and anode excitation, except that in the first case action potentials occur once the current is injected, while in the second case once the current is "broken". Recording is normally obtained by using external electrodes placed close to the nerve membrane (possibly far from the stimulating electrodes) or, in sufficiently large neurons, by inserting an electrode intracellularly and measuring the difference in potential with respect to a reference electrode put outside. As it is in fact not necessary, in order to understand the present thesis, to know the details of how stimulation and recording of action potentials are achieved, this introductory section is concluded here and space is left for deeper discussions in the following chapters on more theoretical aspects of nerve excitation.

to the discussion, we decided to omit it.

CHAPTER II

# Theoretical Foundations of the Hodgkin-Huxley model

As fellows of the Trinity College in Cambridge in the beginning of the 1930s, both Hodgkin and Huxley were strongly influenced, in the formative period of their careers, by the lively scientific environment their university offered in those years. Reading Hodgkin's personal reminescences (Hodgkin 1976, 1983), the impression one gets of the two young scientists is that of two extremely active and curious students: Hodgkin in particular was very dynamic since his early years and used to enjoy reading a considerable amount of articles and books on several scientific arguments, physiology included. It is interesting to notice that, among the literature Hodgkin cites as most formative, there appear the works of Adrian, Hill, Rushton, Lillie, and Lucas; all of whom were or had been fellows of the Trinity College. There is little doubt that, for the young Hodgkin as well as for Huxley, entering in direct contact with icones of neurophysiology such as the ones just mentioned, was very motivating.

## 2.1 Premises to the sodium hypothesis

In the early 30s (as in our days) there was, at Cambridge as in most of other Universities where neurophysiology was taught, the common belief that ions where the only charged particles in living tissues whose movement could cause

the generation of an electrical signal. Such idea of the existence of an ionic basis for the phenomenon of nerve excitation can be traced back to the end of the XIX century, after the acceptation among scientists of van t'Hoff 's theory of osmosis in solutions (van t'Hoff 1887), of the hypothesis of dissociation of salts into ions by Arrhenius (Arrhenius 1887), and of the dilution law by Ostwald (Ostwald 1888). The latter scientist in particular was one of the most influential supporters of the concept of semipermeable membrane and among the firsts to suggest for it a role of primary importance in a wide range of biological phenomena, nerve and muscle excitation included. The words Ostwald used in 1890 are very eloquent on this point:

*"It is perhaps not too bold to conjecture that through the properties of the semipermeable membrane discussed here an explanation could be found not only for electrical current in muscles and nerves but also for the puzzling effects of electric fish in particular*" (Ostwald 1890, p.80)

Although the existence of semipermeable membranes was first proposed by Ostwald in 1889, it was his student and collaborator Nerst who, nine years later, opened the way for quantitative explanations of nerve electrical phenomena on the basis of the alteration of ion concentrations induced by externally applied electric currents. Nernst's famous equation

$$V = \frac{RT}{F} \log \frac{[C]_i}{[C]_o}$$

became later the foundation on the basis of which Bernstein proposed in 1902 that the observed potential across nerve membranes in their resting state was due essentially to a high permeability to $K^+$ ions and a low permeability to other ion species, in particular to the macroscopic negative ones, which were known already at the time to be present inside cells. Excitation, on the other hand, was suggested to originate from a loss in membrane selectivity leading to the unification of the positive ions of the extracellular space with the just mentioned intracellular negative ones. Under this perspective, the potential across the nerve membrane was expected to approach zero during excitation as a consequence of charge neutralization (Bernstein 1902, 1912).

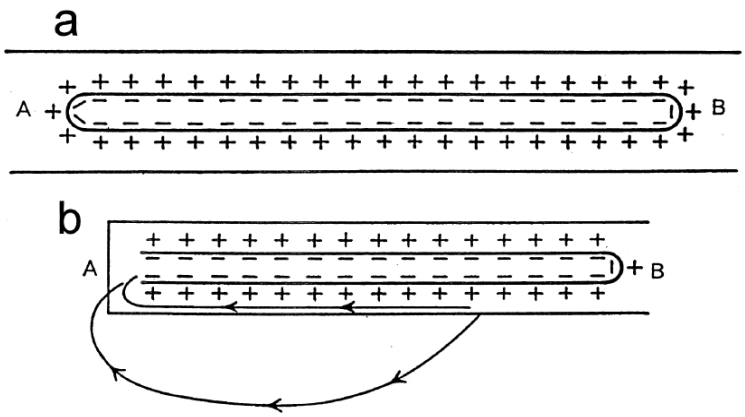


Figure 1.1. Bernstein's theory of membrane polarization. (A) At resting conditions the negative charges inside and the positive ones outside are separated. (B) Following injury (specifically following the removal of part of the membrane), positive and negative ions bind leading to cell depolarization. According to Bernstein, during excitation a similar phenomenon of unification of charges takes place. Reproduced from (Bernstein 1912) via (Piccolino1998).

It should be noted that the theory just mentioned was the last of several theories conceived by Berstein during his life and in fact the only one that was attributed to his name in the years that followed. Although in this theory the possibility that during excitation the membrane potential could reverse sign (as we know nowadays) was formally denied, the German scientist was not at all unaware of that. It was indeed he himself the one who, not yet thirty, recorded for the first time in history the profile of a negative membrane depolarization wave (see Fig 1.2 top). Why the theory Bernstein proposed some thirty-fourty years after these recordings does not allow their explanation still remains unclear. One plausible interpretation is that the failure of obtaining qualitatively similar results from muscle preparations - note that (Fig 1.2) is obtained from a frog nerve and not from a muscle - might have dissuaded him from relying on these results in his last comprehensive book Elektrobiologie (Grundfest 1965), where a modified version of (Fig 1.2 top) now lacking an overshoot appeared (Fig 1.2 bottom) together with the following sentence:

"*Eine Konsequenz dieses Theorie würde nun sein, dass die negative Schwankung eine maximale Grenze erreichen müsste, welche durch die Stärke des Membran-*

*potentials gegeben wäre, und das dieser bei der Reizung sich nicht umkehren könnte*" (Bernstein 1912, p 105)

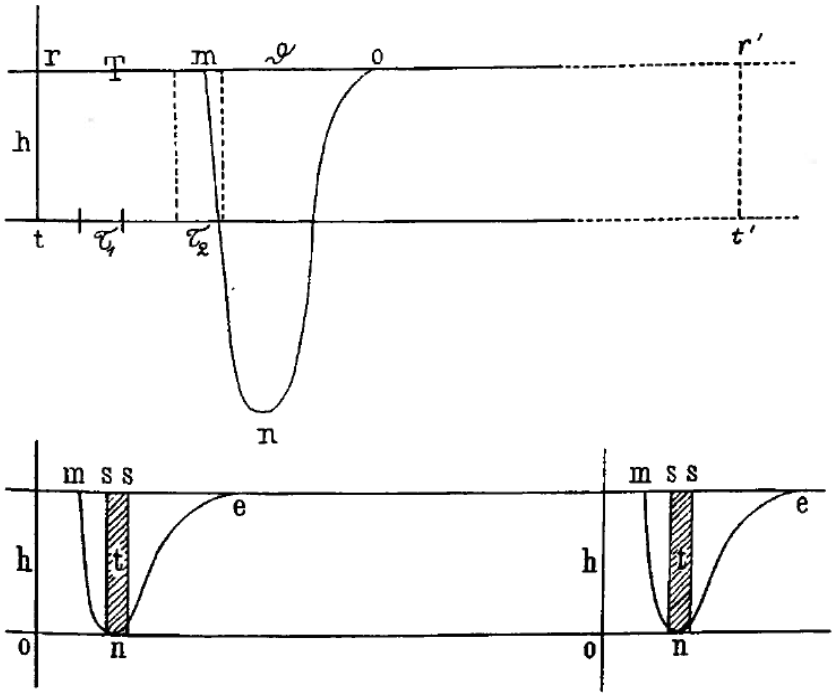


Figure 1.2. Top: The first published recording of nerve depolarization (from Bersntein 1868). $m$ and $n$ represent the current which can be seen to exceed more than two-fold the amplitude of the resting current level $h$. The abscissa represents the time, with $\tau_1$ and $\tau_2$ showing different intervals of recording after delivery of the electrical stimulus at time $t$. Bottom: same as top but with no negative variation; from (Bernstein 1912).

Between the end of the nineteenth century and the beginning of the twentieth, the existence of an action potential overshoot was still largely doubted. Bernstein himself being responsible for that, the work (Bernstein 1968) remained poorly cited over the following decades and was overshadowed by the late hypotheses (Bernstein 1902, 1912, Grundfest 1965).

As mentioned before, Bernstein's last theory of nerve resting membrane potential and excitation remained the most popular theory of nerve physiology for

over thirty years; suitable experimental techniques to test it properly however lacked until the late 30s.

During the 30s, a revolution in experimental neurophysiology took place: working on the anatomical structures of squids between the Marine Biological laboratories of Naples, Plymouth, and Woods Hole, the zoologist and physiologist Young identified giant nerve fibers which, after a couple of years of work since the discovery, were demonstrated to be capable to conduct action potentials: the squid giant axon was discovered (Young JZ 1936, 1938). Its extraordinarily large diameter (up to several hundreds of micrometers), together with the easiness to isolate it, allowed for the first time in history accurate electrophysiological studies of single neurons. In 1939 Hodgkin and Huxley published the first trace of an action potential recorded from the squid giant axon using an intracellular electrode (Fig 1.3). Interestingly, contrary to the expectations deriving from Bernstein's last theory, the polarization of the axonal membrane turned out to undergo a significant reverse in sign upon electrical stimulation. The long-standing hypothesis of the German physiologist of the absence of overshoots had to be dismissed, although an explanation for the large positive peak in the voltage trace still could not be provided.

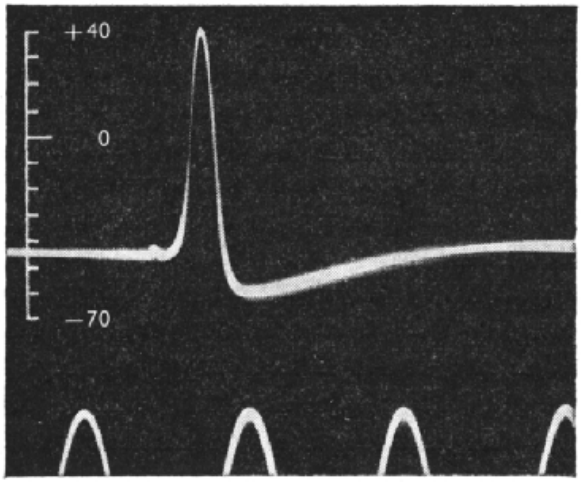


Figure 1.3. First published intracellular recording of an action potential from a squid giant axon. A clear overshoot of 40 mV c.a. can be seen. Reproduced from (Hodgkin and Huxley 1939).

## 2.2 The sodium hypothesis

In 1939 the World War II begun and both Hodgkin and Huxley left the laboratories to work for the army. When they returned in Cambridge to continue their research in 1945, no significant advancements in the understanding of the phenomenon of nerve excitation had been made. The problem was then investigated again from the point where it had been abandoned, until finally Hodgkin and Katz published, in 1949, an hypothesis that was going to become one of the most fundamental assumptions of the neurosciences: the $Na^+$ hypothesis (Hodgkin and Katz 1949). By systematically varying the concentration of sodium in the bathing medium of squid axons, the two found a "reasonable agreement" between the recorded action potential amplitude and the theoretical predictions deriving from Nernst's equation applied to sodium ions only (see Fig 1.4); this observation led them to write the following statement:

*"The reversal of membrane potential during the action potential can be explained if it is assumed that permeability conditions of the membrane in the active state are the reverse of those in the resting state. The resting membrane is taken to be more permeable to potassium than sodium, and the active membrane more permeable to sodium than potassium"*

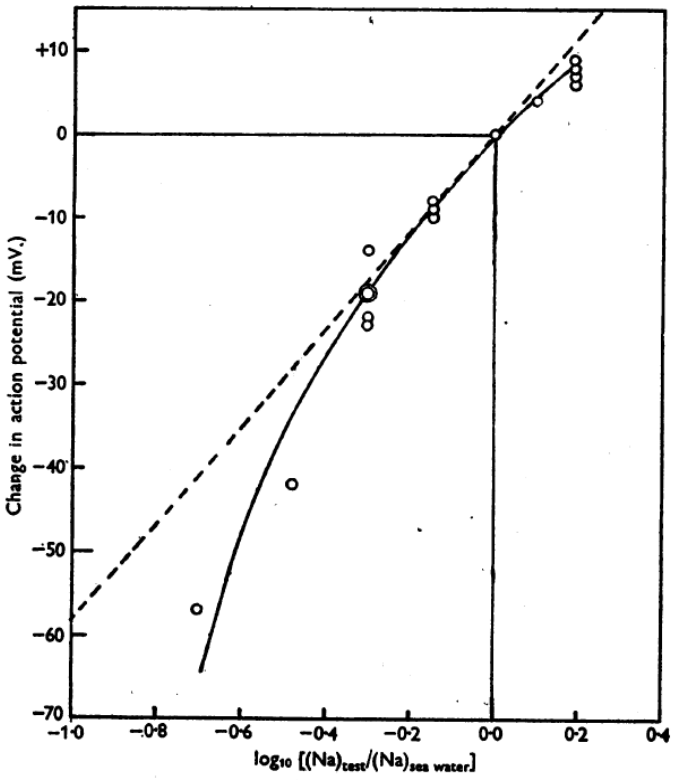


Figure 1.4. Changes in the amplitude of the action potential upon variations of the extracellular concentration of sodium. The dotted line is the theoretical prediction calculated using Nernst's equation; the open circles are the experimental results. The ordinata represents the difference between the transmembrane voltage in the presence of external sea water and of given altered $Na^+$ concentration, according to: $\Delta V = V_{test} - V_{seawater} = \frac{RT}{F} \log \frac{[Na_{seawater}]}{[Na_{test}]}$. Reproduced from (Hodgkin and Huxley 1949).

The belief in the validity of the $Na^+$ hypothesis was reinforced after Hodgkin's student Keynes managed to show that nerve excitation induced an increase in the transmembrane flow of sodium ions by tracing the movement of the radioactive isotope Na24 in repeatedly stimulated squid axons (Keynes 1949, 1951). It should be noted here that these experiments allowed to investigate only very long timescales (minutes to hours) and by no mean could resolve single millisecond action potentials. More precise measurements of the movement of sodium was claimed to be possible after the invention by Marmont and Cole of current

and voltage-clamp techniques respectively (Marmont 1949, Cole 1949). It is worth making few precisations on these latter techniques too to avoid misunderstandings.

Both current and voltage clamp are techniques of electrical stimulation and recording which, through the use of a system of electrodes with feedback on the stimulating electrode, allow to control and eventually keep constant either the current or the voltage across the nerve membrane while recording its response. This gives, in fact, only impedance measurements and does not tell anything about the mechanism behind them. In particular, there is no reason to assume that the electrical signals recorded come from the movement of ions, nor especially from the movement of sodium.

Establishing in fact *a priori* that sodium and potassium were responsible for the impedance measurements coming from current and voltage clamping the squid giant axon, Hodgkin, Katz, and Huxley extended their hypotheses in the beginning of the 50s and claimed to have managed to separate the contribution of the two ions in the process of nerve excitation. As it will be seen in the following chapters, it is on this never properly tested basis[2] that the famous mathematical model was conceived in 1952.

[2]There exists in fact experimental evidence that contradicts the sodium hypothesis in a wide variety of preparations, the squid giant axon included (see Chapter IV).

## 2.3 Quantitative models before 1952

Before entering into the details of the quantitative models that either directly or indirectly influenced the work of Hodgkin and Huxley, a short premise is due.

As mentioned in the previous section, intracellular recordings became possible only in the late '30, after the discovery of the squid giant axon by Young. No detailed voltage trace showing the now well known phenomenon of overshoot thus existed before Hodgkin's and Huxley's publication (Hodgkin and Huxley 1939). Not surprisingly, the purpose of the quantitative models conceived during the first three or four decades of the twentieth century was to describe (or sometimes even explain) how an externally applied electrical stimulus could lead to excitation, rather than the characteristic temporal variation of the transmembrane potential that we are nowadays used to think about.

Even extracellular recordings were in fact not very common; rather, muscle twitches were often taken as criterion to establish whether nerve excitation had been successfully elicited or not. Essentially until Hodgkin and Huxley, thus, the most important test for the validity of a model was the comparison with the experimentally found relationships between the applied electrical stimulus and the time required to induce excitation - i.e. the so called strength-duration relation -.

The first model of this historical treatment will be Nernst's model (Nernst 1899, 1908). There exist, of course, quantitative models of nerve excitation which were worked out before the German physicist had published anything on the topic. The impact of Nernst's theory on the neuroscientific community was however so high that it shaded all previous attempts to quantitative modeling. As Lapicque wrote in his classic book of neurophysiology "L'excitation en fonction du temps" published in 1926,

*"All the modern physiology, when it made the effort to build up a physical theory of electrical excitation, took Nernst's theory as starting point*" (Lapicque 1926, p. 141)

Although Nernst's model was soon recognized to be untenable, the hypothesis adopted that ionic movements are to be regarded as the only cause of nerve excitation became the foundation for most of the theories to come, the one published by Hodgkin and Huxley in 1952 included .

### 2.3.1 Nernst (1899 - 1908)

By the end of the nineteenth century, the inefficiency of high frequency current (∼10 KHz) to stimulate nerves was an established fact. A theoretical explanation for it was however still lacking. Under the hypothesis that "*a galvanic current can in organic tissue (a purely electrolitic conductor) only cause the movement of ions, i.e. concentration changes, and nothing else*" (Nernst 1904), Nernst gave an interpretation of the phenomenon mentioned in terms of accumulation of salts[3] in the vicinity of semipermeable membranes (polarization).

The German physicist assumed the presence of two membranes permeable only to some of the salts dissolved in the physiological solution and sufficiently far from each other to be considered at infinite distance. Under the influence of an externally applied electric field, the salts to which the membrane was permeable would guarantee the passage of current, while the non-permeating ones would accumulate, the whole process of accumulation always occurring under the opposing tendency of re-equilibration by diffusion. Nernst focused then on one non-permeating (unspecified) salt, assuming the onset of nerve excitation to depend exclusively on its concentration at a given distance from one of the two membranes. Let this latter membrane be at position $x = 0$ ; call $c$ the concentration of the salt, $D$ its coefficient of diffusion. The process just explained is formalized as follows:

$$c_t = Dc_{xx}$$

$$c_x(0,t) = -\frac{k}{D}i(t)$$

$$c(x,0) = c_0 \qquad \text{for} \quad 0 \leq x < \infty$$

where $i$ is the applied current density, $k$ a proportionality constant. Excitation would occur when $c(\bar{x}) - c_0 \geq A$, with $\bar{x}$ any fixed distance from the membrane where accumulation of $c$ takes place and $A$ positive constant.

The solution of this problem can be derived in the following manner (Strauss 2007):

Let $v(x,t) = c(x,t) + x\frac{k}{D}i(t)$ , then

[3]Note that, in order to stress the electroneutrality condition, Nernst used the term "salts" and not "ions" in his works, as often wrongly reported by other authors when treating his theories.

$$v_t - Dv_{xx} = x\frac{k}{D}\frac{di}{dt}$$

$$v_x(0,t) = 0$$

$$v(x,0) = c_0 + x\frac{k}{D}i(0) \qquad \text{for} \quad 0 \le x < \infty$$

The method of even extension to the whole line can now be used, by defining the new function $u$ such that

$$u_t - Du_{xx} = f(x,t) := \begin{cases} x\frac{k}{D}\frac{di}{dt} & x > 0 \\ 0 & x = 0 \\ -x\frac{k}{D}\frac{di}{dt} & x < 0 \end{cases}$$

and

$$u(x,0) = \phi(x) = \begin{cases} c_0 + x\frac{k}{D}i(0) & x > 0 \\ c_0 & x = 0 \\ c_0 - x\frac{k}{D}i(0) & x < 0 \end{cases}$$

Since $u$ is even and the extension of $v$ , then $u_x(0,t) = 0$ and $u(x,t) = v(x,t)$ for $x > 0$. The solution for the inhomogeneous problem in $u$ is:

$$u(x,t) = \int_{-\infty}^{\infty} S(x-y,t)\phi(y)dy + \int_0^t \int_{-\infty}^{\infty} S(x-y,t-s)f(y,s)dyds$$

where

$$S(x,t) = \frac{1}{2\sqrt{\pi D t}} e^{-\frac{x^2}{4Dt}}$$

is the diffusion kernel. Thus, $v$ is given by

$$\begin{aligned} v(x,t) = &\int_0^{\infty} \left[S\left(x-y,t\right) + S\left(x+y,t\right)\right]\left[\phi(y)\right]dy \\ &- \int_0^t \int_0^{\infty} \left[S(x-y,t-s) + S(x+y,t-s)\right]\left[-y\frac{k}{D}i'(s)\right]dyds \end{aligned}$$

After substitution of $v$ into $v(x,t) = c(x,t) + x\frac{k}{D}i(t)$, the solution for $c$ is obtained:

$$c(x,t) = \int_0^\infty [S(x-y,t) + S(x+y,t)] \left[c_0 + y\frac{k}{D}i(0)\right] dy$$
$$- \int_0^t \int_0^\infty [S(x-y,t-s) + S(x+y,t-s)] \left[-y\frac{k}{D}i'(s)\right] dyds - x\frac{k}{D}i(t)$$

i.e.

$$c(x,t) = \int_0^\infty \left[\frac{1}{2\sqrt{\pi Dt}}e^{-\frac{(x-y)^2}{4Dt}} + \frac{1}{2\sqrt{\pi Dt}}e^{-\frac{(x+y)^2}{4Dt}}\right] \left[c_0 + y\frac{k}{D}i(0)\right] dy$$

$$- \int_0^t \int_0^\infty \left[\frac{1}{2\sqrt{\pi D(t-s)}}e^{-\frac{(x-y)^2}{4D(t-s)}} + \frac{1}{2\sqrt{\pi D(t-s)}}e^{-\frac{(x+y)^2}{4D(t-s)}}\right] \left[-y\frac{k}{D}i'(s)\right] dyds - x\frac{k}{D}i(t).$$

Nernst calculated the explicit form of the solution for both sinusoidal and constant currents. In the case of sinusoidal currents of the form $i = I\sin nt$, where $n$ and $a$ are constants being respectively amplitude and frequency of the stimulus, this reads

$$c(x,t) = c_0 + \int_0^t \int_0^\infty \left[\frac{1}{2\sqrt{\pi D(t-s)}}e^{-\frac{(x-y)^2}{4D(t-s)}} + \frac{1}{2\sqrt{\pi D(t-s)}}e^{-\frac{(x+y)^2}{4D(t-s)}}\right] * \quad (1)$$

$$* \left[y\frac{k}{D}nI\cos nt\right] dyds - x\frac{k}{D}I\sin nt.$$

For constant currents, on the other hand, the explicit solution is

$$c(x,t) = c_0 + \frac{k}{D}I\left[\frac{\sqrt{4Dt}}{\sqrt{\pi}}e^{-\frac{x^2}{4Dt}} + x\int_{-\infty}^{x}\frac{1}{2\sqrt{\pi Dt}}e^{-\frac{z^2}{4Dt}}dz - x\int_x^{+\infty}\frac{1}{2\sqrt{\pi Dt}}e^{-\frac{z^2}{4Dt}}dz\right] - x\frac{k}{D}I$$

meaning

$$c(x,t) = c_0 + \frac{k}{D}I\left[\frac{\sqrt{4Dt}}{\sqrt{\pi}}e^{-\frac{x^2}{4Dt}} - 2x\int_x^{+\infty}\frac{1}{2\sqrt{\pi Dt}}e^{-\frac{z^2}{4Dt}}dz\right]. \quad (2)$$

By considering the concentration of salts at $x = 0$, (1) and (2) reduce respectively to

$$c(0,t) = c_0 + \frac{k}{D}\int_0^t nI\cos ns\frac{\sqrt{4D(t-s)}}{\sqrt{\pi}}ds$$

and

$$c(0,t) = c_0 + 2kI\sqrt{\frac{t}{\pi D}}.$$

Now, the first solution gives, upon change of variables and integration,

$$c(0,t) = c_0 + \frac{I}{\sqrt{n}}\frac{2k}{\sqrt{\pi D}}\left[\sin nt\int_0^{\sqrt{nt}}\cos\left(y^2\right)dy - \cos nt\int_0^{\sqrt{nt}}\sin\left(y^2\right)dy\right]$$

For sufficiently long timescales, the two integrals in the square brackets can then be approximated by the limit value $\frac{\sqrt{2\pi}}{4}$ so to give

$$c(0,t) = c_0 + \frac{I}{\sqrt{n}}\frac{k}{2\sqrt{D}}\left(\sin nt - \frac{\pi}{4}\right).$$

Nernst reached in this way the conclusion that the strength-duration relation for the critical change of salt concentration in eliciting nerve excitation had to be of the form $\frac{I}{\sqrt{n}}$ =constant for alternating currents and $I\sqrt{t}$ =constant for constant currents.

An extensive comparison between the theoretical results and the experimental data was made: for high frequency currents ($\geq 100$ Hz c.a.) the agreement turned out to be excellent; for constant currents, however, the predictions could only partially be satisfied. In particular, while according to Nernst any constant current, independently of its stength, would have sooner or later induced a nerve response, experiments showed unequivocably that this was not the case for sufficiently weak currents.

*

The rigorous framework provided by Nernst's theory revealed to be extremely attractive to physically acquainted neuroscientists since the very first publication. As just mentioned, however, Nernst himself, in discussing his results, pointed out that some experimental observations could not be given an explanation if his equations were to be used. The tentative to give a physical basis for the phenomenon of nerve excitation was further pursued by Hill,

who calculated the changes in the concentration of ions for membranes at short distance apart, instead of infinite as Nernst had suggested (Hill 1910). This modified model led to a formula of the form

$$i = \frac{\lambda}{1 - \mu\theta^t} \tag{3}$$

$i$ being the applied current, $\lambda$, $\mu$ and $\theta$ constants whose value could be (at least in principle) deduced from electrophysiological experiments [4]. Equation (3) was found to better describe the experimental findings, in particular the ones deriving from the application of long stimuli and implying accomodation .

A qualitatively different perspective was investigated by the French physiologist Lapicque, who developed a model that was going to be regarded in the years that followed as "the simplest and most generally useful model of nerve excitation" (Cole, page 122) : the resistance-capacity electric circuit model [5].

### 2.3.2 Lapicque (1907 - 1926)

In 1907 Lapicque published a quantitative theory of nerve excitation based on the analogy with the circuit in Figure 1.21. This circuit is composed of

(i) a resistance $R$, representing the sum of the resistances in the stimulating circuit, the intrinsic resistance of the portion of nerve interposed between the electrodes, and the local membrane resistance at the anode;

(ii) a capacitor $K$, representing the capacitance of the nerve membrane

(iii) a resistance $\rho$, representing the leakage resistance of the portion of membrane in contact with the cathode. It is worth noting that this resistance was assumed by Lapicque to be very small, for it corresponded to the flow of ions to which the membrane was considered to be largely impermeable.

[4] I don't derive here the equation (3), as a more general approach to the problem nerve excitation by Hill himself - including also the model from 1910 as a special case - will be treated in the following pages.

[5] Lapicque worked also on an hydraulic model, which he conceived in the tentative to explain qualitatively the phenomenon of accomodation. As this model had however far minor resonance than the electric circuit one, I have omitted its treatment in the present work. For details, reference is made to (Lapicque 1926).

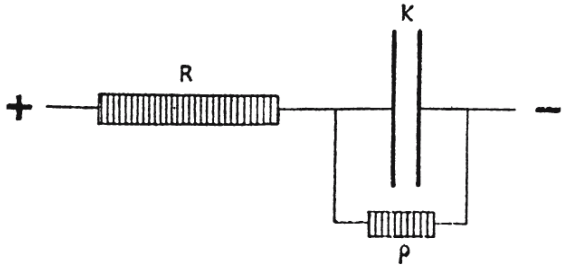


Figure 1.21: Lapicque's electric circuit model. $R$ and $\rho$ are resistances, $K$ a condenser. Reproduced from (Lapicque 1907).

Lapicque mentioned more than once that his model constituted only an approximation; this clarified, its capability to describe satisfactorily several of the experimental results that he and his colleagues neurophysiologists had obtained so far, was emphasized.

Calling $V$ the potential difference between the two extrema of the circuit and $v$ the one across the capacitor, then the infinitesimal charge increment at $K$ is (using a physical notation) given by

$$Kdv = \frac{V-v}{R}dt - \frac{v}{\rho}dt \tag{4}$$

where $\frac{V-v}{R}$ is the current through $R$ and $\frac{v}{\rho}$ the current through $\rho$. Rearrangement of (4) gives

$$-\frac{R+\rho}{KR\rho}dt = \frac{dv}{v - \frac{V\rho}{R+\rho}}$$

which has general solution

$$C - t\frac{R+\rho}{KR\rho} = \log\left(\frac{V\rho}{R+\rho} - v\right).$$

Under the initial condition $v(0) = 0$, $C = \log\frac{V\rho}{R+\rho}$ and

$$e^{-t\frac{R+\rho}{KR\rho}} = 1 - \frac{R+\rho}{V\rho}v.$$

The strength of the applied voltage can then be explicited as a function of time:

$$V = v\frac{R+\rho}{R}\frac{1}{1-\exp\left(-t\frac{R\rho K}{R+\rho}\right)}$$

or

$$V = \frac{\alpha}{1 - e^{-\frac{t}{\beta}}} \tag{5}$$

where $\alpha = v\frac{R+\rho}{R}$ , $\beta = \frac{R\rho K}{R+\rho}$.

Assuming $K$, $\rho$, and $v$ constant, being moreover $R$ known, Lapicque could estimate experimentally the value of the parameters $\alpha$ and $\beta$ by simply exposing the nerves to two different stimulations and using the equation(s) (5). The validity of the strength-duration relationship (5) could then be directly tested for any other electrical stimulus. Although Lapicque's predictions were confirmed to a major extent, some unresolved questions were left. In particular, the inefficiency of slowly increasing currents to elicit excitation still could not be given a formal explanation.

*

The tentatives to describe the process of nerve excitation by using physically grounded approaches, for example by referring to laws governing the movement of ions (Nernst, Hill) or to the analogy with electric circuits (Lapicque), proved incapable to provide a satisfactory explanation the wide variety of experimental observations available, unless the formulation of ad hoc assumptions far from having a physiological meaning was made. Not surprisingly, all the efforts in using existing physical laws soon appeared senseless.

In 1932, Blair published the first purely abstract model of nerve excitation, i.e. a model that was openly not inspired to any physical phenomenon. This model was going to influence determinantly the attitude underlying the most popular quantitative descriptions of nerve activity that were to be produced in the following decade.

### 2.3.3 Blair 1932

On purely abstract grounds, Blair defined a variable $p$ generically referred to as the "state of excitation", whose temporal variation was directly proportional to

the applied exciting current or voltage $V$, and whose tendency to return to the resting value was proportional to its own magnitude. In mathematical terms, $p$ was made satisfying the ordinary differential equation

$$\frac{dp}{dt} = KV - kp \tag{6}$$

where $K$ and $k$ are constants. Excitation was then assumed to occur when $p$ reached a threshold value $h$ that, from the comparison with the experimental observations, was deduced to be best represented by a linear function of the applied stimulus, i.e. a function of the form $h = \overline{h} + \alpha V$, where $\overline{h}$ and $\alpha$ are constants. Imposing as initial condition $p(0) = 0$, Blair could directly obtain the strength-duration relation for direct currents by integration

$$\int_0^{\overline{h}+\alpha V} \frac{kdp}{KV - kp} = -k \int_0^t dt$$

which gives

$$\log \frac{KV}{KV - k(\overline{h} + \alpha V)} = kt \tag{7}$$

where $t$ is the time necessary to the stimulus to induce excitation.

By the time Blair conceived his model, the concept of rehobase, i.e. the maximum current strength such that its constant application for an infinite period does not cause excitation, had gained popularity in the field of quantitative neurophysiology. Since the rehobase was experimentally measurable, it became common to express strength-duration relations in terms of such quantity in order to test the validity of the theories. For Blair's model, from the definition itself of the rehobase, it follows that this is the current $R$ satisfying the equality

$$KR - k(\overline{h} + \alpha R) = 0$$

i.e.

$$R = \frac{k\overline{h}}{K - k\alpha}.$$

Equation (7) can thus be written in "canonical form" as

$$\log \frac{KV}{(K - \alpha k)V - (K - k\alpha)R} = kt$$

or

$$\log \frac{CV}{V-R} = kt \tag{8}$$

where $C = \frac{K}{K-k\alpha}$.

Blair showed that by a proper choice the parameters it was possible to derive from his model both Lapicque's and Hill's formula (3) for the strength-duration relations [6]:

**Lapicque:** Consider equation (4). To obtain the explicit expression of the variation in time of the charge $q$ at the condenser $C$, one just needs to divide by $dt$, for $\frac{dq}{dt} = C\frac{dV}{dt}$. Thus

$$\begin{aligned} C\frac{dv}{dt} &= \frac{V-v}{R} - \frac{v}{\rho} \\ &= \frac{V}{R} - v\left(\frac{1}{R} + \frac{1}{\rho}\right) \\ &= \frac{V}{R} - v\frac{\rho+R}{R\rho}, \end{aligned}$$

which is Blair's equation (6) with $K = \frac{1}{R}$ and $k = \frac{\rho+R}{RC\rho}$.

**Hill:** Equation (8) can be written as

$$\frac{V}{V-R} = e^{kt-\log C} = Ce^{kt}$$

Now, subtracting and adding $\frac{R}{V-R}$ to the first term, one obtains

$$1 + \frac{R}{V-R} = Ce^{kt}$$

which, after some rearrangement, gives

$$V = \frac{RCe^{kt}}{Ce^{kt}-1} = \frac{R}{1-\frac{e^{-kt}}{C}},$$

that is Hill's formula (8) for $\mu = \frac{1}{C}$ and $\theta = e^{-k}$.

[6] Note that the two formulas are in fact already formally equivalent. The derivation of the two as provided by Blair in the original paper (Blair 1932) is anyway followed here.

Regarding Hill's formula in particular, Blair emphasized that there existed no way to discern between that and his theory on the basis of the observations from direct current stimulation. Given the simplicity of the derivation of Blair's strength-duration equation, this was undoubtedly a remarkable result.

*

Blair's model, combining mathematical simplicity and desctriptive accuracy of the experimental outcomes, attracted the interest of theoretical physiologists, among which the most important are Rashevsky and Hill. The two extended Blair's model to what are known nowadays as the "two factor theories", i.e. theories in which the process of nerve excitation is described formally by the combined dynamics of two variables, one excitatory and one inhibitory.

### 2.3.4 Rashevsky 1933

By the beginning of the twentieth century, Loeb had demonstrated the primary importance of a balanced ratio between monovalent and divalent cations in the bathing medium for the maintenance of nerve activity, evidencing in particular the destabilizing effects of the former ions as opposed to the stabilizing effects of the latter ones.

Inspired by the classical work of the German physiologist, observing that stimulation by means of electric current would have brought to the cathode not only monovalent but also divalent cations, Rashevsky deduced that the process of nerve excitation would have been best described by using two independent variables, which he named $e$ and $i$, being respectively the excitatory and the inhibitory variable. It is worth noting that despite of the several references Rashevsky made in his publications to possible parallelisms between the quantities appearing in the model and the concentrations of different ion species, the physiological basis of the whole theory was only apparent and superficial. The use of physical laws governing the movement of ions was indeed carefully avoided. Instead, $e$ and $i$ were made satisfying, at the cathode, two "Blair-type" equations:

$$\frac{de}{dt} = KI - k(e - e_0) \tag{9}$$

$$\frac{di}{dt} = MI - m(i - i_0) \tag{10}$$

where $I$ is the current, $e_0$ and $i_0$ are the concentrations of monovalent and divalent cations at resting conditions, $K$, $M$, $k$ and $m$ are constants. From the observation that neurons were normally not spontaneously firing, Rashevsky deduced that $e_0$ and $i_0$ had to satisfy the inequality $e_0 < i_0$. Assuming moreover in general higher diffusivity for monovalent cations than for divalent ones, $m$, $k$, $M$ and $K$ were chosen such that $m \ll k$ and $\frac{K}{k} < \frac{M}{m}$. In this framework, excitation would occur once the ratio $\frac{e}{i}$ reached a fixed threshold which, without loss of generality, was taken to be 1.

For a constant current $I$ established at time $t = 0$, one obtains

$$e = e_0 + \frac{KI}{k}(1 - e^{-kt})$$
$$i = i_0 + \frac{MI}{m}(1 - e^{-mt}).$$

It follows that the time $t$ at which excitation takes place has to satisfy the condition

$$e_0 + \frac{KI}{k}(1 - e^{-kt}) = i_0 + \frac{MI}{m}(1 - e^{-mt}). \tag{11}$$

Equation (11) is a transcendental equation and can be only given an approximate solution. Rashevsky derived it for very short time $t$ or for especially small $m$. From these two approximations distinct solutions were obtained, each of which was found to better describe different experimental results.

Small $t$: from the Taylor expansion

$$e^{-xt} = 1 - xt + \frac{x^2t^2}{2} - ...$$

for $e^{-kt}$ and $e^{-mt}$, truncation after the second power and substitution into (11) leads to

$$(Mm - Kk)It^2 - 2(M - K)It - 2(i_0 - e_0) = 0. \tag{12}$$

The necessary condition for the existence of real solutions for this equation, is that

$$(M-K)^2I^2 + 2(Mm-Kk)(i_0-e_0)I > 0$$

i.e.

$$I > \frac{2(Kk-Mm)(i_0-e_0)}{(M-K)^2}.$$

It follows from this that the rehobase is

$$R = \frac{2(Kk-Mm)(i_0-e_0)}{(M-K)^2}. \tag{13}$$

Since the general solutions of (12) are given by

$$t_1 = \frac{(M-K)I - \sqrt{(M-K)^2 - 2(Mm-Kk)(e_0-i_0)I}}{(Mm-Kk)I} \tag{14}$$

$$t_2 = \frac{(M-K)I + \sqrt{(M-K)^2 - 2(Mm-Kk)(e_0-i_0)I}}{(Mm-Kk)I},$$

the strength-duration relation for cathodal stimulation is obtained by substitution of (14) into (12), and reads

$$t_1 = \frac{M-K}{Mm-Kk}\left(1 - \sqrt{1-\frac{R}{I}}\right)$$

Small $m$: consider equation (11). If $m$ is made sufficiently small, $e$ will have reached its asymptotic value $e_0 + \frac{KI}{k}$ before $i_0$ has varied significantly. This means that one could approximate (11) with

$$e_0 + \frac{KI}{k}\left(1 - e^{kt}\right) = i_0.$$

Rearrangement gives

$$kt = \log\frac{KI}{KI - k(i_0-e_0)},$$

i.e. Blair's formula (7). Identical arguments as the ones made for Blair's model apply then to the case small $m$.

The equations treated so far have been restricted to the description of the dynamics at the cathode. Rashevsky studied also the dynamics at the anode,

assuming similar equations as (9-10) to hold during stimulation, the only difference being the reversed sign of $I$. To obtain excitation at break, the initial conditions were first fixed at the stationary values that $e$ and $i$ attained after exposure of the nerve to a continuous current for sufficiently long time, meaning

$$e_0' = \lim_{t\to\infty}\left[e_0 - \frac{KI}{k}\left(1-e^{-kt}\right)\right] = e_0 - \frac{KI}{k} \quad (15)$$

$$i_0' = \lim_{t\to\infty}\left[i_0 - \frac{MI}{m}(1-e^{-mt})\right] = i_0 - \frac{MI}{m}. \quad (16)$$

Upon opening the circuit, no external current is delivered to the nerve anymore, i.e. $I = 0$. This means that the equations to be considered are reduced to

$$\begin{aligned}\frac{de}{dt} &= -k\,(e-e_0')\\ \frac{di}{dt} &= -m\,(i-i_0')\end{aligned}$$

which, after substitution of (15,16) give

$$\begin{aligned}\frac{de}{dt} &= -KI - k\,(e-e_0')\\ \frac{di}{dt} &= -MI - m\,(i-i_0')\,.\end{aligned}$$

Following a procedure similar to the one just described for cathodal excitation, Rashevsky obtained also the expressions for anodal rehobase $R_a$ and strength-duration relation, respectively

$$R_a = \frac{2(Kk-Mm)(i_0-e_0)}{(M-K)^2 + 2(Kk-Mm)\left(\frac{M}{m}-\frac{K}{k}\right)}$$

and

$$t = 3.41\left[\frac{M-K}{Mm-Kk}\left(1-\sqrt{\frac{1}{2}}\right) - t_a + 3.41\sqrt{1-\frac{R_a}{I}}\right],$$

where

$$t_a = 3.41\frac{M-K}{Mm-Kk}\left(1-\sqrt{\frac{1}{2}}\right) + 0.293\frac{\sqrt{(M-K)^2 - 2(Mm-Kk)\left(\frac{M}{m}-\frac{K}{k}\right)}}{Mm-Kk}.$$

Given the possibility to derive both rehobase and excitation time from the experimental results, the predictions of the model could finally be tested with the available data obtaining very good agreement.

*

Few years after Rashevsky proposed his theory, apparently not being aware of that, Hill published a model which was, at least in its fundamental aspects, identical to the one of the Russian biophysicist. It is worth going in some detail into Hill's approach to understand to which extent the two models are similar and in which (apparent) aspect they differ. Hill's treatment is moreover of historical importance, as the British scientist was one of the most brilliant and influential figures in the twentieth-century nerve physiology. His choice to avoid the use of explicit physical laws as the ones he himself had assumed to hold some twenty-five years before, is especially significant of a spread still-far-from-clear understanding of the origin of nerve excitation.

### 2.3.5 Hill 1936

In an extensive work published in 1936 in the Journal of Physiology (Hill 1936), Hill proposed a formal description of nerve excitation using two variables, which he named $V$ and $U$ , respectively referred to as "local potential" and threshold. Although these two variables were in fact inspired by the long investigated quantities in neurophysiology, the parallelism was exploited by the British scientist only to the extent to provide a guideline for the derivation of the mathematical equations. In particular, as in Blair's and Rashevsky's models, there was no tentative to bind neither $V$ nor $U$ to any specific biophysical process.

To derive a formal mathematical description for the dynamics of $V$ and $U$ under the influence of a stimulating current, Hill started from some basic but fundamental observations over the phenomenon of accomodation: it was long known by the mid '30s that slowly rising currents induced a gradual rise in the threshold, up to the point that stimulation could become ineffective when the

gradient of its increase was below a certain value. Similarly to Blair, Hill thought that this behaviour (accomodation) reflected a variation in time of the threshold $U$ itself, and that this variation had to be a consequence of the altered physico-chemical condition of the nerve, generically referred to in his model as the "local potential". Under this perspective, both $V$ and $U$ would have been influenced by the externally applied current, although in a different way: the first directly, the second indirectly. From the experimental observations available so far, Hill deduced moreover $V$ as well as $U$ to have a natural tendency to return to their resting value, the timescale of the relaxation being however much longer for the latter than for the former quantity.

The simplest equations to describe the observations made turned out to be

$$\frac{dV}{dt} = bI - \frac{V - V_0}{k} \tag{17}$$

$$\frac{dU}{dt} = \frac{V - V_0}{\lambda} - \frac{U - U_0}{\beta} \tag{18}$$

here $I$ is the externally injected current, while $b$ , $\beta$ , $\lambda$ and $k$ are constants with $\lambda$ and $k$ satisfying $\lambda \gg k$ . It follows moreover without saying that excitation would occur once $V$ equals $U$.

To simplify the mathematical analysis of his model, Hill assumed the condition $\beta = \lambda$ to hold [7]. In the most general case then, admitting any form of current, the solution of (17-18) was given by

$$V = V_0 + be^{-\frac{t}{k}} \int_{\theta=0}^{\theta=t} Ie^{\frac{\theta}{k}} d\theta \tag{19}$$

$$U = U_0 + \frac{e^{-\frac{t}{\lambda}}}{\lambda} \int_{\theta=0}^{\theta=t} (V - V_0)e^{\frac{\theta}{\lambda}} d\theta \tag{20}$$

Now, several kinds of stimulations were treated in (Hill 1936); of these, by far the most interesting for the comparison with the experimental results as well as with the previously published models, are cathodal and anodal constant currents. I report them both here below.

Cathode excitation:

[7] Note that there exists in fact no objective physiological parallelism for this choice.

In the case of constant currents, (19-20) become

$$V = V_0 + bkI\left(1 - e^{-\frac{t}{k}}\right) \tag{21}$$

$$U = U_0 + bkI\left[1 + \frac{e^{-\frac{t}{k}}}{\frac{\lambda}{k} - 1} - \frac{e^{-\frac{t}{\lambda}}}{1 - \frac{k}{\lambda}}\right]. \tag{22}$$

The condition of excitation then translates into

$$V_0 + bkI\left(1 - e^{-\frac{t}{k}}\right) = U_0 + bkI\left[1 + \frac{e^{-\frac{t}{k}}}{\frac{\lambda}{k} - 1} - \frac{e^{-\frac{t}{\lambda}}}{1 - \frac{k}{\lambda}}\right]$$

i.e.

$$\begin{aligned}\frac{V_0 - U_0}{bk} &= I\left[e^{-\frac{t}{k}} + \frac{ke^{-\frac{t}{k}}}{\lambda - k} - \frac{\lambda e^{-\frac{t}{\lambda}}}{\lambda - k}\right] \\ &= I\frac{\lambda}{\lambda - k}\left(e^{-\frac{t}{k}} - e^{-\frac{t}{\lambda}}\right),\end{aligned}$$

which, after rearrangement, gives

$$I = \frac{\lambda - k}{\lambda bk}\frac{V_0 - U_0}{e^{-\frac{t}{k}} - e^{-\frac{t}{\lambda}}}. \tag{23}$$

Equation (23) is the strength-duration relation for constant current stimuli. Hill derived also its compact form in terms of the rehobase, after having calculated the latter according to the definition by imposing $V = U_0$ at $t = \infty$ in (21). This giving $bkI_0 = U_0 - V_0$, substitution into (23), led to

$$I = \frac{I_0\left(1 - \frac{k}{\lambda}\right)}{e^{-\frac{t}{\lambda}} - e^{-\frac{t}{k}}}. \tag{24}$$

It is worth noting that under the condition $\lambda \gg k$ mentioned above, equation (24) reduces to

$$I = \frac{I_0}{1 - e^{-\frac{t}{k}}}$$

which is the formula Hill had proposed in 1910 and that had become famous since then for its accuracy in fitting a wide variety of experimental observations.

Anode excitation:

To obtain the strength-duration relation at the anode, Hill assumed that upon opening the stimulating circuit after exposure of the nerve to an externally applied constant current over a duration $\bar{t}$ , a current of opposite intensity is produced. Considering that at the anode the current is of reversed sign with respect to that at the cathode, this means that equations (21) and (22) take the form:

$$V = V_0 - bkI\left(1 - e^{-\frac{t}{k}}\right) + bkI\left(1 - e^{-\frac{t-\bar{t}}{k}}\right) = V_0 - bkI\left(e^{-\frac{t-\bar{t}}{k}} - e^{-\frac{t}{k}}\right)$$

$$U = U_0 - bkI\left[1 + \frac{e^{-\frac{t}{k}}}{\frac{\lambda}{k} - 1} - \frac{e^{-\frac{t}{\lambda}}}{1 - \frac{k}{\lambda}}\right] + bkI\left[1 + \frac{e^{-\frac{t-\bar{t}}{k}}}{\frac{\lambda}{k} - 1} - \frac{e^{-\frac{t-\bar{t}}{\lambda}}}{1 - \frac{k}{\lambda}}\right]$$

$$= U_0 - bkI\left[\frac{e^{-\frac{t-\bar{t}}{\lambda}} - e^{-\frac{t}{\lambda}}}{1 - \frac{k}{\lambda}} - \frac{e^{-\frac{t-\bar{t}}{k}} - e^{-\frac{t}{k}}}{\frac{\lambda}{k} - 1}\right]$$

Anode excitation is then expected to occur when

$$V_0 - bkI\left(e^{-\frac{t-\bar{t}}{k}} - e^{-\frac{t}{k}}\right) = U_0 - bkI\left[\frac{e^{-\frac{t-\bar{t}}{\lambda}} - e^{-\frac{t}{\lambda}}}{1 - \frac{k}{\lambda}} - \frac{e^{-\frac{t-\bar{t}}{k}} - e^{-\frac{t}{k}}}{\frac{\lambda}{k} - 1}\right]$$

i.e. when

$$U_0 - V_0 = bkI\frac{\lambda}{\lambda - k}\left[e^{-\frac{t-\bar{t}}{k}} - e^{-\frac{t}{k}} - e^{-\frac{t-\bar{t}}{\lambda}} + e^{-\frac{t}{\lambda}}\right]$$

or

$$I = \frac{U_0 - V_0}{bk}\frac{\left(1 - \frac{k}{\lambda}\right)}{e^{-\frac{t-\bar{t}}{k}} - e^{-\frac{t}{k}} - e^{-\frac{t-\bar{t}}{\lambda}} + e^{-\frac{t}{\lambda}}}.$$

In the discussion of his paper, Hill very honestly remarked the limitations of his abstract mathematical treatment which, despite of its capability to describe the electrical behaviour of many physiological preparations, was still far from providing a satisfactory explanation of the process of nerve excitation. It is

worth reporting Hill's own words in conclusion of this section:

"*The statement given above of the two time-factors in electric excitation is the simplest possible one, and it is realized only too clearly that in certain aspects it is inadequate.* [...] *It might be regarded as a further weakness that no physical model has been proposed. The surface of the nerve has not been supposed to act as an electrical condenser; the "local potential" has not been identified with the electrotonic potential; the concentration of ions at a semipermeable membrane has not been assumed to determine excitation; the constituents of a sensitive surface have not been imagined to flow, or alter their shape, under the influence of a current, and so to lead to "accomodation".* [...] *No specific physical or chemical theory is offered of the nature of the "local potential" V, of "threshold" U, or of their time constants k and* $\lambda$ *. Their behaviour is only discussed.*"

*

After the publication of the two factor theories of nerve excitation by Hill and Rashevsky, a considerable amount of mathematicians entered the field of Neuroscience, some by proposing brand new (always abstract) approaches, some by extending the work of the two biophysicists. Among these it is worth mentioning a group of mathematicians from the University of Chicago who, among the contributions given, formally demonstrated the equivalence of the two models just treated (see Appendix for details) (Offner F 1937, Young G 1937, Householder AS 1939, 1944).

Despite of the popularity the two factor theories gained between the 30s and the 40s, their final contribution to the advancement of the understanding of nerve activity was objectively inexistent. The most important questions regarding the physico-chemical processes involved in the phenomenon of action potential were indeed left not only unanswered but untackled.

It happened that in 1939 Hodgkin and Huxley obtained the first intracellular recording of the action potential from a squid giant axon. Their publication (Hodgkin and Huxley 1939) was a true revolution in the field. Specifically, for what concerns the approaches to quantitative theories, the reliability, precision and reproducibility of the recordings caused a paradigm shift from the purpose of modelling only the processes that lead to nerve excitation, to that of describing the phenomenon itself too.

Still, however, even in the mid 40s, there was no real clue of what could be the effective mechanisms underlying action potentials. This general state of ignorance is brilliantly reflected in an unfortunately poorly cited work by Hodgkin and Huxley (Hodgkin and Huxley 1945), where the two advanced a variety of plausible alternatives to explain the experimental results they themselves obtained from squids. Although not extensively quantitative, it is worth going through this publication before analysing the famous model published in 1952; interestingly, it will be noticed that no reference to sodium ions was made at the time[8].

### 2.3.6 Hodgkin and Huxley 1945

After the end of the second World War, Hodgkin and Huxley repeated and extended the experiments performed in 1939 on resting and action potentials in the squid giant axon (Hodgkin and Huxley 1945); in the section dedicated to the discussion of the results, the two analyzed four qualitatitatively different explanations that could account for the phenomenon of nerve excitation. These are:

(i) The onset of a selective permeability of the membrane towards the anions in the axoplasm

(ii) A change in the orientation of dipoles present in the membrane surface

(iii) The existence of an inductive element in the membrane

(iv) In series membrane capacity and electromotive force

(i) The first explanation derived from the observation that depolarization could be produced in principle not only by an inward flow of cations, but by an outward flow of anions as well. Although plausible, the idea of an increased mobility of negative particles was subsequently considered unlikely due to the fact that the contributions of $K^+$ and $Cl^-$ to the membrane potential were believed too high to be overcomed in this way.

(ii) With the second explanation of activity in terms of reorientation of

[8] As mentioned before, the $Na^+$ hypothesis was published only in 1949.

membrane dipoles Hodgkin and Huxley were voluntarily neglecting any direct influence due to the movement of ions.

Starting from the hypothesis that the cell surface was largely composed of a double layer of lipid molecules arranged so to have a negative polar hydrophilic headgroup facing the exterior of the membrane and an hydrophobic hydrocarbon chain facing the interior [9], Hodgkin and Huxley reasoned that the negatively charged headgroups could contribute little to the resting potential due to the symmetry of the bilayer or, assuming asymmetric conditions, due to the reequilibration of the ion species free to transverse the membrane. At the same time, however, they considered that *"a transient wave of negativity would occur if the inner layer of dipoles were removed, or deorientated in some way when the membrane was excited"*. The plausibility of this hypothesis was tested with a quantitative model based on the electrical circuit in figure (1.5). Referring to it, $E$ represents the resting electromotive force, $R_4$ the membrane resistance, $C$ the capacity, $\psi$ the voltage due to the oriented dipoles, $V_m$ the total potential difference across the membrane, $I_m$ the current. This last was then given by

$$I_m = \frac{V_m - E}{R_4} + \frac{Cd(V_m - \psi)}{dt}$$

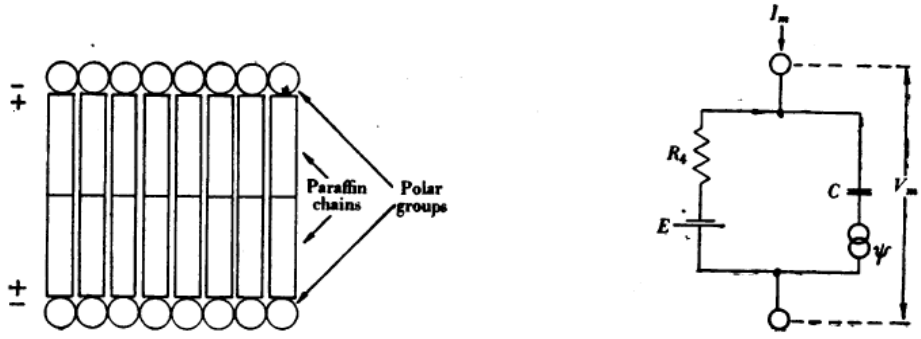


Figure 1.5: (a) Representation of the membrane as a lipid bilayer; (b) electrical circuit model for the electrical behaviour of the neuronal membrane.

Considering that at the peak of activity $\frac{dV_m}{dt} = 0$ and substituting to $V_m - E$, $R_4$, $I_m$ and $C$ the values obtained from the experimental observations available

[9] Note that Danielli's hypothesis revealed later to be essentially correct.

(see Cole and Curtis 1939), Hodgkin and Huxley estimated $\frac{d\psi}{dt}$ at the crest of the action potential to be $-4.2 \cdot 10^3 V \cdot sec^{-1}$. "*This result indicates that the rate of change of molecular orientation would be equivalent to that produced by a dipole layer with a potential difference of* 420 $mV$ *collapsing during a period of* 0.1 *msec. This is not an impossible assumption, although it is a little hard to imagine that such a change would leave the membrane capacity unaltered*" [10]

(iii) As third explanation, Hodgkin and Huxley considered the hypothesis of the existence of an inductive element in the neuron. This hypothesis had been originally proposed by Cole in 1941 (Cole 1941) and assumed an analogy between the electrical behaviour of the membrane of the squid giant axon and that of the circuit in Figure (1.6). Again, as in the previous point, $E$ is the resting electromotive force, $R_4$ the membrane resistance, $C$ the (fixed) capacity; $L$ is the inductance, which was suggested to arise from a still non-identified piezoelectric element in the membrane. Hodgkin and Huxley tested the idea by subsituting to the variables just mentioned the values in Figure (1.6b), where the switch and the resistance in series on the right were conceived to account for the observed drastic decrease in transmembrane resistance during excitation. Simulations showed that this third explanation could account for an overshoot of the same order of magnitude of the one normally recorded in living cells; despite of this, no structure in the membrane could be identified with the required piezoelectric characteristics, and the hypothesis was not pursued further.

[10] In fact, it should be noted that the membrane capacity is not at all unaltered upon excitation: an increase of few point percentage magnitude is indeed known to occur during the rising phase of the action potential and a correspondent decrease during the repolarizing one (Cole and Curtis 1939).

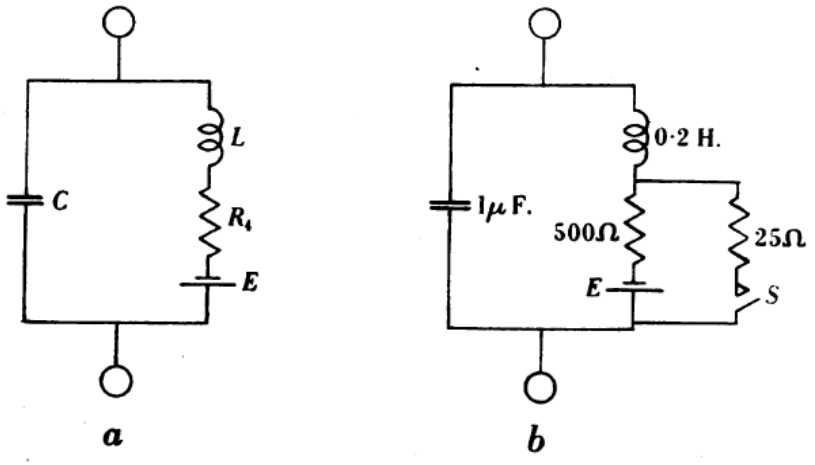


Figure 1.6: (a) Electrical circuit analogue originally adobted by Cole (Cole 1941); (b) same circuit as in (a) with switch and estimated values for the components.

(iv) The fourth explanation consisted in assuming the electromotive force to be in series with the membrane capacity. Figure (1.7) shows the electric analogue of the neuronal membrane conceived according to this hypothesis at resting conditions (a), in the vicinity of an active region (b), and during excitation (c). Neither quantitative nor qualitative investigations of this last explanation were reported in (Hodgkin and Huxley 1945). Its plausibility, in fact only on very general grounds, was however briefly suggested.

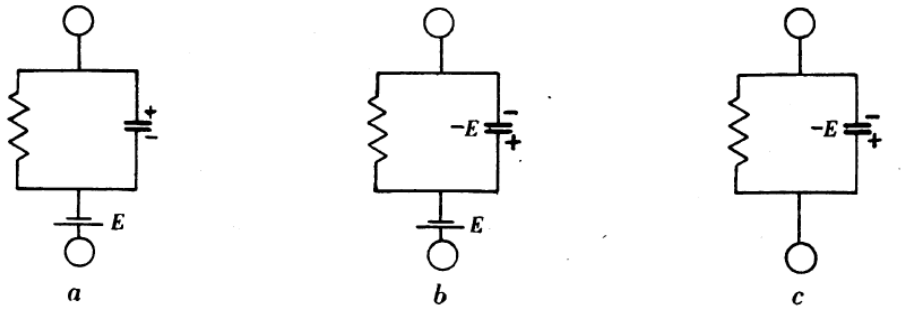


Figure 1.7: Electric circuit model with in-series membrane capacity and electromotive force.

## 2.4 The legacy of the early quantitative models

Before discussing in detail the model Hodgkin and Huxley conceived in 1952 (Chapter III), it is worth spending few words to emphasize the salient features of the approaches to quantitative modelling of nerve excitation discussed so far, as well their impact and relationship with respect to the studies the two physiologists from Cambridge were finally led to several years later.

We showed that already in the beginning of the XIX century, Nernst published a model based on the fundamental assumption that the sole underlying cause of nerve excitation was the movement of ions. The mathematical framework developed by the German physicist was then recalled to consist not surprisingly of a diffusion equation with specific boundary conditions reflecting the presence of an hypothetical semipermeable membrane which could allow ion accumulation in its vicinity (thus the generation of a potential difference). This approach, although sometimes apparently put aside in favour of simpler and easy-to-handle models, remained since then at the basis of the conception of action potentials whenever tentatives of physical interpretation were made.

After having presented Nernst's theory, we discussed another highly influential work in the field of mathematical modelling of nerve excitation: Lapicque's electric circuit model. This latter, conceived in fact even before Nernst had published the final version of his theory, was seen by the most popular physiologists of the mid 1900 - Hodgkin and Huxley included - as the reference work for any physically-inspired theoretical work (see for example Cole 1968). The legacy of Lapicque's work in our current conception of nerve excitation and related phenomena certainly cannot be overestimated: the electrical circuit model, together with Nernst's ionic theory, will be found at the very foundation of the physical interpretation on which Hodgkin and Huxley built their model some half a century later.

Before closing this chapter it seems fair to say that also the abstract models conceived during the 30s, in particular the ones by Blair, Rashevsky, and Hill, had some influence on later works. Indeed, although these models did not strongly and directly influence the one by Hodgkin and Huxley as Nernst's

Lapicque's models did, still it will be possible to recognize their their legacy in the purely abstract definition of the dynamics of the ion-channel gating variables [11]. Moreover, Blair's, Rashevsky's and Hill's efforts towards an analytically treatable model of excitation can be clearly "ritrovati" in perhaps the most popular post- Hodgkin-Huxley model, i.e. the one by FitzHugh and Nagumo (see Chapter V).

[11] Note that Hill was one of Hodgkin's reference figures in Cambridge during the years of his scientific formation (Hodgkin 1976, 1983).

CHAPTER III

# The Hodgkin-Huxley Model

We have seen that by the mid twentieth century the hypothesis of the flow of ions as the one and only cause for voltage variations in nerve cells had been advanced (Nernst); that the idea of using RC electric circuit analogues to model the responses to externally applied electric currents had been proposed (Lapicque); that ordinary differential equations of purely abstract nature had sometimes been used with in fact not surprising success (Rashevsky and Hill). We have moreover seen that in 1945 the understanding of the origin of nerve excitation was still very far from being clear.

In 1949 Hodgkin and Katz published the so-called sodium hypothesis (Hodgkin and Katz 1949; see preceeding Chapter); the idea of independent transmembrane pathways for sodium and potassium was moreover conceived in the few years that followed thanks to the use of the voltage-clamp technique just introduced by Cole (see Historical Background, paragraph 2.2) (Hodgkin and Huxley 1952a, b, c). It is on this theoretical and experimental basis that the famous Hodgkin-Huxley model was proposed in 1952. I report here in detail the model and the most salient aspects behind its derivation, with some references to current interpretations.

## 3.1 Hodgkin and Huxley 1952: the static model

In the model Hodgkin and Huxley proposed in 1952, the behaviour of a nerve fiber is described using an electrical network where the membrane is represented

by a capacitor of fixed capacitance, and the ion pathways through the membrane are represented by three resistance-capacitor modules arranged in parallel (see Fig 2.1). Of such modules, two are ion-specific - one for $Na^+$ and one for $K^+$- while the other is related to leakage phenomena meant to be generated by unspecified ions. Modern *ad hoc* extended versions of the model commonly include ion-specific pathways also for $Ca^{2+}$and $Cl^-$as well as for other ions. However, even if these modifications allow more accurate descriptions of the electrophysiological recordings, no conceptual difference exists between the old and most of the updated versions.

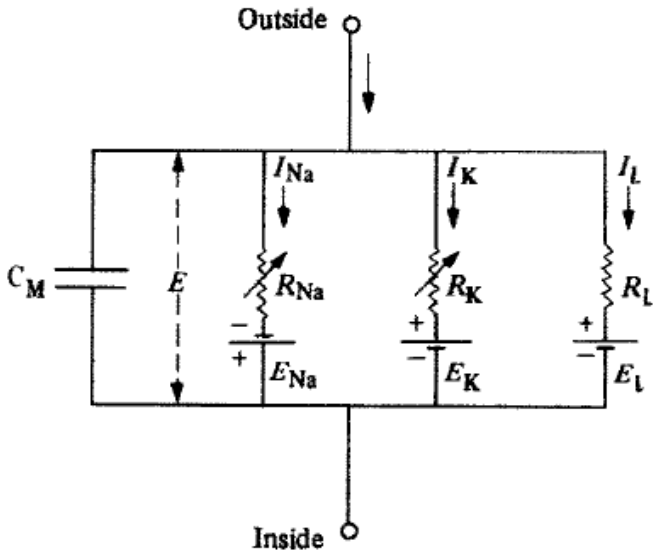


Figure 2.1 The electric circuit analogue used by Hodgkin and Huxley to describe nerve excitation. Reproduced from (Hodgkin and Huxley 1952).

Referring to the circuit above, the total current between the inside and the outside of a nerve cell is given by the sum of both a membrane-capacitive and an ionic component. Thus

$$I = C_M \frac{dV}{dt} + I_i \tag{25}$$

where

$I$ is the total membrane current (inward current positive)

$I_i$ is current density carried by ions (inward current positive)
$V$ is the membrane potential (depolarization negative)
$C_M$ is the membrane capacitance (assumed constant)
$t$ is time

From the parallelism of the ionic currents in the circuit in Figure 2.1, it follows that the term $I_i$ can be further subdivided into the algebraic sum of the current carried by the sodium ions ($I_{Na}$), the one by potassium ($I_K$), and the one by the other unspecified ions ($I_l$). Thus $I_i = I_{Na} + I_K + I_l$. Using now Ohm's law $I = gV$, where $g$ is the conductance (i.e. the reciprocal of the resistance), the three currents $I_{Na}$, $I_K$ and $I_l$ can be expressed respectively as the products

$$I_{Na} = g_{Na}(V - E_{Na})$$

$$I_K = g_K(V - E_K)$$

$$I_l = g_l(V - E_l)$$

In this context the conductance terms $g_i$ $(i = Na, K, l)$ are related to the facility that the correspondent ion species encounter in crossing the neuronal membrane, while $E_{Na}$, $E_K$and $E_l$ are the specific equilibrium potentials calculated, according to the ionic hypothesis of nerve conduction, using Nernst's formula

$$V_i = \frac{RT}{F} \log \frac{[i]_{out}}{[i]_{in}}$$

where, taking the $Na^+$ ion as example, $[Na]_{out}$ represents the concentration of $Na^+$ in the extracellular space, and $[Na]_{in}$ is it's concentration inside.

Hodgkin and Huxley assumed $E_{Na}$, $E_K$ and $E_l$ to be constant, while $g_{Na}$ and $g_K$ to be function of time and membrane potential. As a consequence, the derivation of a dynamic equation for the conductances $g_{Na}$ and $g_K$ was required. The procedure the two physiologists followed is given here below. Although it might be more intuitive to treat $g_{Na}$ before $g_K$ since it was the movement of $Na^+$ ions to be believed to be responsible for the generation of the action potential, the opposite order will be followed as originally done in (Hodgkin and Huxley 1952).

### 3.2.1 The potassium conductance

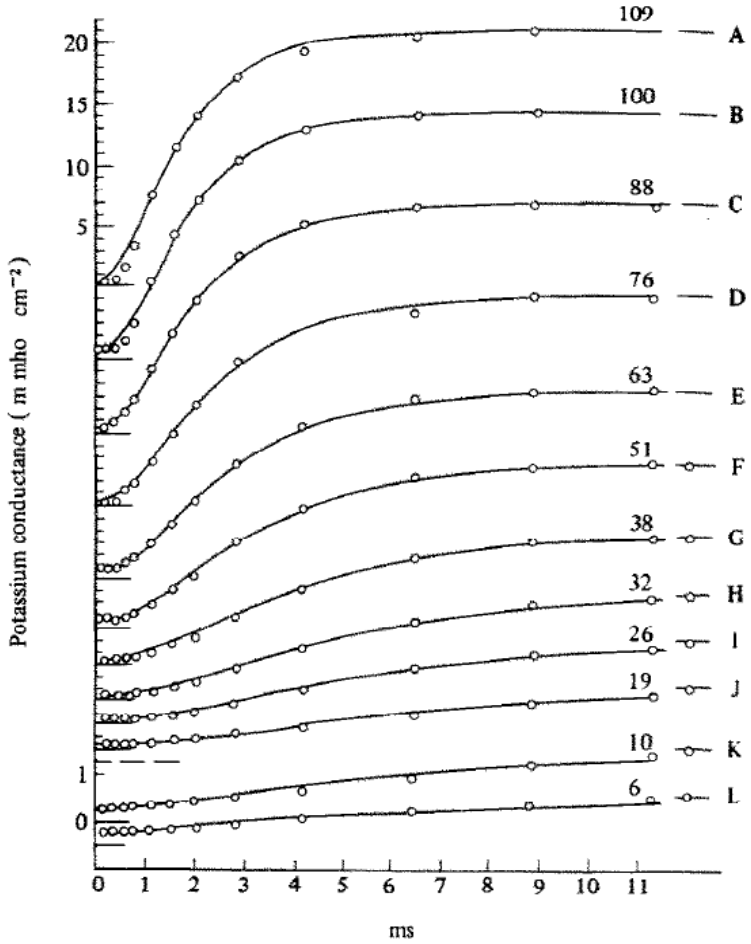


Figure 2.2 Variation of the potassium conductances $g_K$ measured after establishing abruptly a transmembrane voltage equal to the numbers on the right. The empty circles are the potassium conductances recorded, while the continue curves are the ones calculated from eq (26)-(27).

In Figure 2.2, the empty circles represent the potassium conductances deduced from electrophysiological recordings Hodgkin and Huxley performed on the squid giant axon at different voltage steps. Given the results obtained, the

two physiologists proposed the following equations with the intent to fit the data acquired:

$$g_k = \bar{g_k} n^4 \tag{26}$$

$$\frac{dn}{dt} = \alpha_n(1-n) - \beta_n n \tag{27}$$

where $\bar{g_k}$ is a constant representing the maximum potassium conductance (experimentally estimated; dimensions conductance/cm$^2$), $\alpha_n$ and $\beta_n$ are voltage-dependent time-independent rate constants (dimensions of $t^{-1}$), while $n$ is a scalar variable $\in [0, 1]$.

It is stressed that equations (26) and (27) were choosen among many possible solutions which could fit the data in Fig 2.2 equally well and that they lack any physical basis. At the same time, it is worth noting that in their famous paper from 1952, Hodgkin and Huxley observed a posteriori that such equations would agree with an hypothetical mechanism of transport of $K^+$ ions through the membrane based on the cooperativity of four unspecified similar components. Nowadays, these four components are commonly identified with the well known tetrameric structure of the so-called $K^+$ channel (Hille 2001), where the concomitant activation of the four voltage sensing domains (VSD) opens a central cavity through which the $K^+$ ions are thought to flow driven by the electrochemical potential gradient across the membrane. Under this perspective, $n$ is often interpreted as the proportion of VSD in the active state, $1-n$ as the proportion of inactive VSD, $\alpha_n$ as the rate of activation and $\beta_n$ as the rate of inactivation.

In order to obtain the fitting curves displayed in Figure 2.2, the voltage dependency of both the rate constants $\alpha_n$ and $\beta_n$ needed to be specified. Moreover, an initial condition $n_0$ had to be given. Hodgkin and Huxley proceeded then in the following way: first $n_0$ and the corresponding solution of (26) and (27) in terms of $\alpha_n$ and $\beta_n$ were derived, and then the expression of these latter two was explicited.

During the voltage-clamp experiments of (Fig 2.2), the membrane potential was initially clamped at a resting level $V_m = 0$, and only afterwards it was abruptly brought to the predefined value shown on the right in the figure (numbers). When $V_m$ is still equal to zero, the initial condition for $n$ can be written in terms of the initial values of $\alpha_{n0}$ and $\beta_{n0}$, where the index 0 here stands for

$V = 0$. By substitution in () it is obtained that

$$n_0 = \frac{\alpha_{n0}}{\alpha_{n0} + \beta_{n0}}$$

and that the solution of (27) corresponding to such an initial condition will have the form

$$n(t) = n_\infty - (n_\infty - n_0)e^{-\frac{t}{\tau}}$$

where $n_\infty = \frac{\alpha_n}{\alpha_n + \beta_n}$ is the value that $n$ eventually attains after the voltage step, and $\tau = \frac{1}{\alpha_n + \beta_n}$ is the time-scale of the process; $\alpha_n$ and $\beta_n$ are respectively the values of $\alpha$ and $\beta$ after the new voltage is set. In order to permit the comparison with the experimental data, the expression for the potassium conductance was explicited in the form

$$g_K = \{(g_{K\infty})^{\frac{1}{4}} - [(g_{K\infty})^{\frac{1}{4}} - (g_{K0})^{\frac{1}{4}}] \exp(-\frac{t}{\tau_n})\}^4 \tag{28}$$

where $g_{K\infty}$ and $g_{K0}$ are respectively the value that $g_K$ has after the voltage step and at time $t = 0$. In this way the estimation of both the quantities $n_\infty$ and $\tau_n$ from the best fit could be achieved. Since $\alpha_n$ and $\beta_n$ can be written as

$$\alpha_n = \frac{n_\infty}{\tau_n}$$

$$\beta_n = \frac{1 - n_\infty}{\tau_n}$$

for each voltage clamped, their value could be directly deduced from the estimates of $n_\infty$ and $\tau_n$ obtained from eq (28) and the data in Figure 2.2. For each voltage tested in the electrophysiological experiments, correspondent values for $\alpha_n$ and $\beta_n$ could be derived and the explicit expression deduced by applying the same procedure of best fit (Fig 2.3). Specifically, for the experimental conditions under which Hodgkin and Huxley worked, the following results were obtained (Hodgkin and Huxley 1952):

$$\alpha_n = 0.01(V + 10)/[\exp \frac{V + 10}{10} - 1] \tag{29}$$

$$\beta_n = 0.125 \exp(V/80) \tag{30}$$

This completely determined the expression of the potassium conductance.

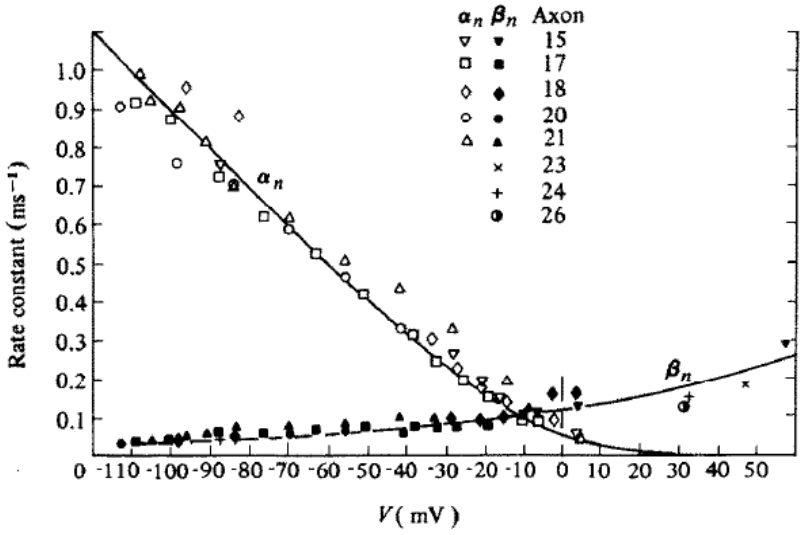


Figure 2.3: Rate constats $\alpha_n$ and $\beta_n$ related respectively to the rise and fall of the potassium conductance. The symbols are the estimates for the correspondent specific axon used during the experiment (labeled with numbers). The continuous lines are the curve-fitting.

### 3.2.2 The sodium conductance

For the derivation of the sodium conductance, Hodgkin and Huxley adopted a similar approach as for the potassium conductance. Since however the dynamics of the former was found to be qualitatively different from the one of the latter (see Figure 2.4), a new function had to be chosen in order to fit the data. Among the possibilities available, the use of two variables each of which obeying a first-order equation was preferred by the two physiologists due to its simplicity. Specifically, the following equations were used:

$$g_{Na} = m^3 h \bar{g_{Na}} \tag{31}$$

$$\frac{dm}{dt} = \alpha_m(1-m) - \beta_m m \tag{32}$$

$$\frac{dh}{dt} = \alpha_h(1-h) - \beta_h h \tag{33}$$

where $\bar{g}_{Na}$ is a constant representing the maximum sodium conductance, while $\alpha_i$ and $\beta_i$ $(i = m, h)$ are as before voltage-dependent time-independent rates. $m$ and $h$ play the same role as $n$ for the potassium conductance, respectively for the activation and inactivation of $g_{Na}$.

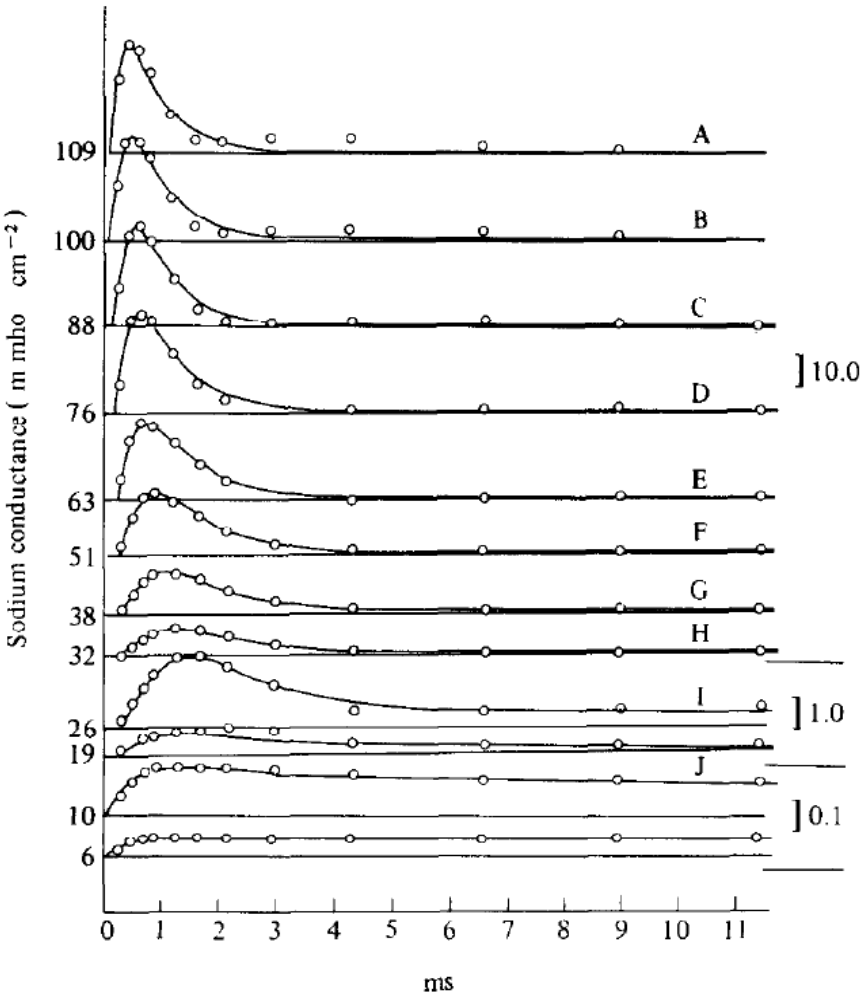


Figure 2.4: Same as Figure 2.2 but for sodium conductance. The voltage steps are shown on the left. Reproduced from (Hodgkin and Huxley 1952).

Again, before proceeding with the specification of the unknown terms, it is worth noting the a posteriori interpretation that was given to the equations (31)-(33). It was suggested that a physical interpretation would have been possible if a mechanism of transport of $Na^+$ ions based on the activation of three similar "molecules" and the non-inactivation of a different one existed. Nowadays, the so called voltage-gated sodium channels - i.e. the proteins that are commonly believed to be related to the voltage-dependent $Na^+$ flow through the lipid bilayer - are known to be constituted by four membrane-spanning monomers and an intracellular protuberance named "activation gate" (Hille 2001). Even accepting the ion-channel hypothesis, which anyway still lacks an objective proof, this time no parallelism can be traced with the molecular level.

The deduction of $\alpha$'s and $\beta$'s expressions follows from the same reasoning used for the potassium rate constants, with the only difference that now there are two first order differential equations rather than only one.

Given the initial resting conditions $m_0$ and $h_0$, the equations for $m$ and $h$ are

$$m(t) = m_\infty - (m_\infty - m_0)e^{-\frac{t}{\tau_m}}$$

$$h(t) = h_\infty - (h_\infty - h_0)e^{-\frac{t}{\tau_h}}$$

where

$$m_\infty = \alpha_m/(\alpha_m + \beta_m) \quad \tau_m = 1/(\alpha_m + \beta_m)$$

and

$$h_\infty = \alpha_h/(\alpha_h + \beta_h) \quad \tau_h = 1/(\alpha_h + \beta_h)$$

Hodgkin and Huxley observed that both $m_0$ and $h_\infty$ could be neglected. The sodium conductance was then approximated by

$$g_{Na} = \bar{g}_{Na} m_\infty^3 h_0 [1 - \exp(-t/\tau_m)]^3 \exp(-t/\tau_h).$$

This last equation was used to fit the data of Figure 2.4 so that for each different voltage step tested during the experiments, the best estimates of $\tau_m$ and $\tau_h$ were deduced. From these, the correspondent values of $\alpha_i$ and $\beta_i$ $(i = m, h)$ could be

obtained (symbols in Figure 2.5) using the relationships

$$\alpha_m = m_\infty/\tau_m \quad , \qquad \beta_m = (1 - m_\infty)/\tau_m$$

and

$$\alpha_h = h_\infty/\tau_h \quad , \qquad \beta_h = (1 - h_\infty)/\tau_h$$

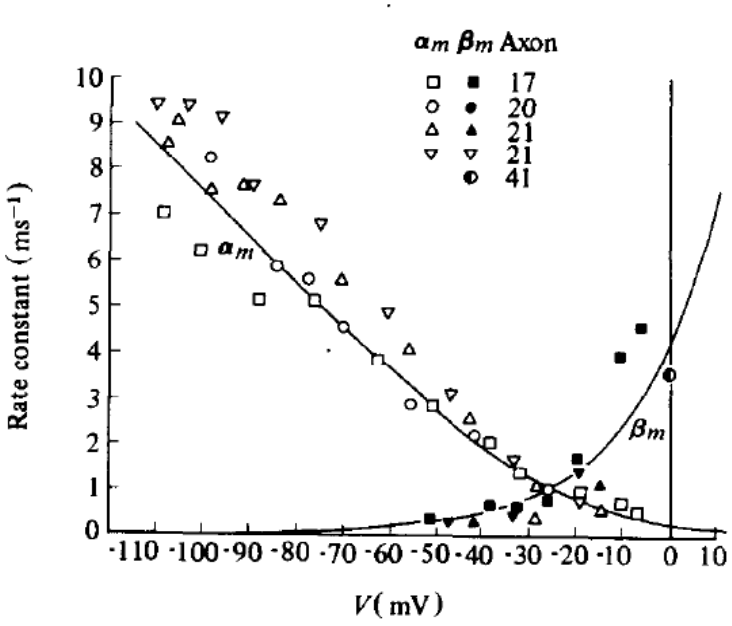


Figure 2.5a: Similar to Figure 2.3 but for the rate constants of activation of sodium conductance.

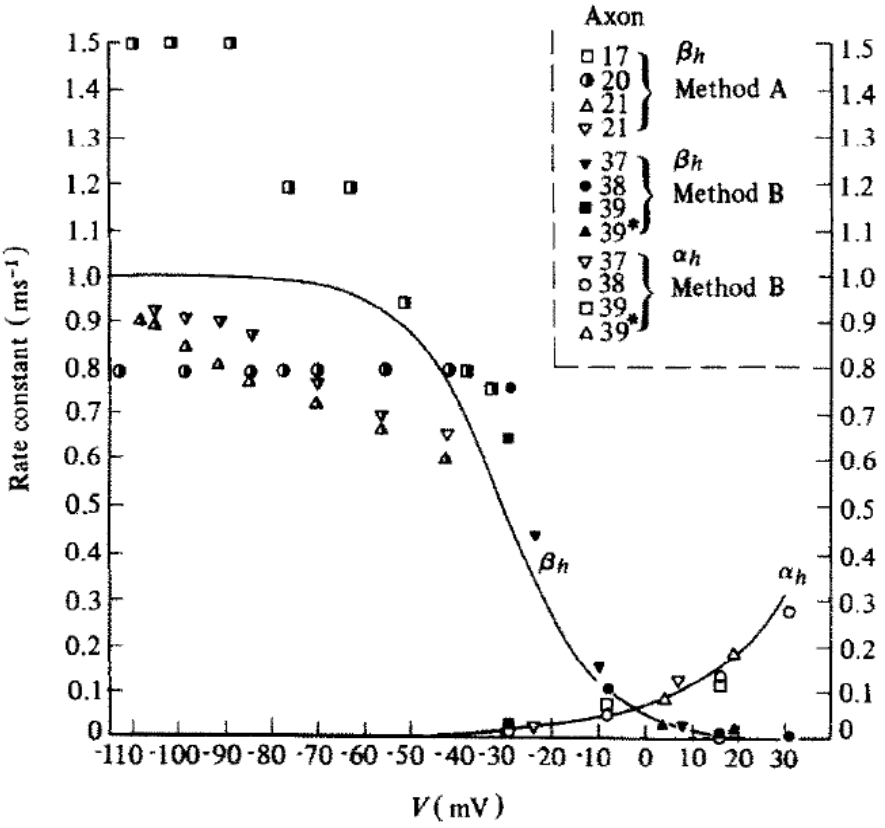


Figure 1.25b: Similar to Figure 1.23 but for the rate constants of inactivation of the sodium conductance. "Methods" appearing on the top-right corner are not relevant in this context, as they are only meant to distinguish between different sources of data.

Iteration of the fitting paradigm to the new data leads to the voltage-dependent expressions of $\alpha_m$, $\beta_m$, $\alpha_h$, $\beta_h$

$$\alpha_m = 0.1(V+25)/(\exp\frac{V+25}{10}-1) \quad \beta_m = 4\exp(V/18) \tag{34}$$

$$\alpha_h = 0.07\exp(V/20) \quad \beta_h = 1/(\exp\tfrac{V+30}{10}+1) \tag{35}$$

In this way the expression of the sodium conductance too was specified.

It is now possible to write the equations that give the time and voltage dependencies of the transmembrane current density of a nerve fiber:

$$I = C_M \frac{dV}{dt} + \bar{g}_K n^4 (V - E_K) + \bar{g}_{Na} m^3 h (V - E_{Na}) + \bar{g}_l (V - V_l) \qquad (36)$$

where

$$\frac{dn}{dt} = \alpha_n (1 - n) - \beta_n n$$

$$\frac{dm}{dt} = \alpha_m (1 - m) - \beta_m m$$

$$\frac{dh}{dt} = \alpha_h (1 - h) - \beta_h h$$

and

$$\alpha_n = 0.01(v + 10)/[\exp \frac{V + 10}{10} - 1]$$

$$\beta_n = 0.125 \exp(V/80)$$

$$\alpha_m = 0.1(V + 25)/(\exp \frac{V + 25}{10} - 1)$$

$$\beta_m = 4 \exp(V/18)$$

$$\alpha_h = 0.07 \exp(V/20)$$

$$\beta_h = 1/(\exp \frac{V + 30}{10} + 1)$$

As usual, potentials are given in m$V$, current densities in $\mu A/\text{cm}^2$, conductances in mmho/cm$^2$, capacity in $\mu F/\text{cm}^2$, and time in msec. Moreover, it is worth repeating that the expressions of $\alpha$'s and $\beta$'s were all derived under specific experimental conditions, in particular at a fixed temperature of $6.3^o$C. Application of the equations at different temperatures requires then proper rescaling.

## 3.2 The propagating action potential

The model explained so far is that of a uniform membrane potential, meaning that it describes the response to an applied stimulus of a neuronal membrane - specifically the one of the squid giant axon - considering the potential as if it were uniform at every instant across the whole surface. Under such a static perspective, the fundamental peculiarity of nerve excitation, i.e. the spreading of the action potential along the nerve, cannot be taken into account.

The extension of the static model to the dynamical one was obtained by Hodgkin and Huxley by referring to the already long known cable theory (Taylor 1963, Rall 1977).

Consider Figure 2.6. The neuron is divided longitudinally in subsections (patches) of length $\Delta x$ . For each of these subsections, the representation introduced in the previous paragraph with in-parallel capacitor and resistances is adopted. $V_i(x)$ and $V_e(x)$ represent respectively the potentials inside and outside the cell at position $x$ , which implies the transmembrane potential to be $V_i(x) - V_e(x)$ . The total current flowing through a membrane patch of length $\Delta x$ is given by $I_m(x)\Delta x$ , where $I_m(x)$ stands for the membrane current per unit length. $I_i(x)$ and $I_e(x)$ , $r_i(x)$ and $r_e(x)$ are the longitudinal internal and external currents and resistances (per unit length).

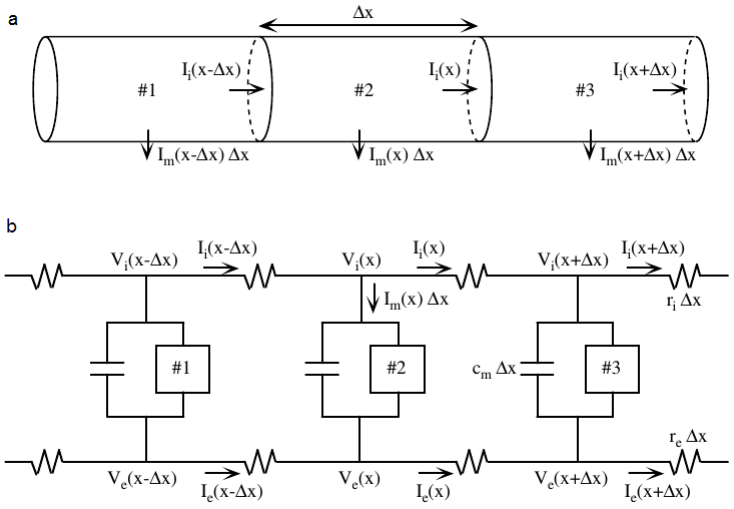


Figure 2.6 (a) Cilindric cable representation of the neuronal axon. (b) Electric circuit equivalent a the membrane: the elements at the top are meant to lay at the

intracellular space, the ones at the bottom to be extracellular, the local Hodgkin-Huxley type circuits #1, #2, #3 to be at the boundary.

From Ohm's law one has

$$V_i(x) - V_i(x+\Delta x) = I_i(x) r_i \Delta x \qquad , \qquad V_e(x) - V_e(x+\Delta x) = I_e(x) r_e \Delta x$$

which give, by taking the limit for $\Delta x \to 0$ ,

$$\lim_{\Delta x \to 0} \frac{V_i(x) - V_i(x+\Delta x)}{\Delta x} = \frac{\partial V_i}{\partial x} = -r_i I_i(x) \qquad , \qquad \frac{\partial V_e}{\partial x} = -r_e I_e(x). \quad (37)$$

At the same time, the conservation of currents at intracellular and extracellular nodes gives

$$I_i(x-\Delta x) - I_i(x) = I_m(x)\Delta x \qquad , \qquad I_e(x-\Delta x) - I_e(x) = -I_m(x)\Delta x$$

meaning, for $\Delta x \to 0$ , respectively

$$\frac{\partial I_i}{\partial x} = -I_m(x) \qquad , \qquad \frac{\partial I_e}{\partial x} = I_m(x).$$

By differentiating equations (37), it is obtained that

$$\frac{\partial^2 V}{\partial x^2} = \frac{\partial^2 (V_i - V_e)}{\partial x^2} = -r_i \frac{\partial I_i}{\partial x} + r_e \frac{\partial I_e}{\partial x} = (r_i + r_e) I_m$$

implying, from the static model derived in the previous paragraph,

$$I_m = c_m \frac{\partial V}{\partial t} + \bar{g}_K n^4 (V - E_K) + \bar{g}_{Na} m^3 h (V - E_{Na}) + \bar{g}_l (V - V_l)$$

where constants and variables are as defined previously. The following equation is then obtained:

$$\frac{1}{(r_i + r_e)} \frac{\partial^2 V}{\partial x^2} = c_m \frac{\partial V}{\partial t} + \bar{g}_K n^4 (V - E_K) + \bar{g}_{Na} m^3 h (V - E_{Na}) + \bar{g}_l (V - V_l) \quad (38)$$

which is the cable equation specific for the Hodgkin-Huxley model.

The two physiologists assumed the squid giant axon to be surrounded by a large volume of conducting fluid [12], which implies $r_e$ to be negligible compared to $r_i$ , thus

$$\frac{1}{(r_i + r_e)} = \frac{1}{r_i} = \frac{a}{2R_i} \quad (39)$$

[12] It should be noted that this assumption is not at all justified, as the squid axon is known to be surrounded by a continuous sheath of glial cells as close as 10 nm to the neuronal surface, which significantly alters the movement of ions close to the membranes.

where $a$ is the radius of the nerve and $R_i$ the specific resistance of the axonal intracellular space. Substituting eq (39) into (38), one obtains

$$\frac{a}{2R_i}\frac{\partial^2 V}{\partial x^2} = C_M\frac{dV}{dt} + \bar{g}_K n^4(V - E_K) + \bar{g}_{Na}m^3h(V - E_{Na}) + \bar{g}_l(V - V_l)$$

Although this equation is not solvable as it is, experimental evidence suggests that one can impose the constraint that the action potential has to travel at constant velocity $\theta$ as well as that its shape is manatained unaltered during the propagation. Substituting the travelling wave ansatz $V(x,t) = \hat{V}(x - \theta t)$ gives

$$\frac{a}{2R_2\theta^2}\frac{d^2V}{dt^2} = C_M\frac{dV}{dt} + \bar{g}_K n^4(V - E_K) + \bar{g}_{Na}m^3h(V - E_{Na}) + \bar{g}_l(V - V_l) \quad (40)$$

Equation (40) together with

$$\frac{dn}{dt} = \alpha_n(1 - n) - \beta_n n$$

$$\frac{dm}{dt} = \alpha_m(1 - m) - \beta_m m$$

$$\frac{dh}{dt} = \alpha_h(1 - h) - \beta_h h$$

are often referred to as the complete Hodgkin-Huxley equations for the propagating action potential.

CHAPTER IV

# Criticism on the sodium hypothesis

As explained in the previous chapters, according to the hypothesis conceived by Hodgkin and Katz in 1949 (Hodgkin and Katz 1949), action potentials are generated by an inward flow of sodium ions from the extracellular space to the intracellular one. In particular, it is assumed that this movement is the one and only responsible for the reversal of membrane polarization during excitation. We will demonstrate in this chapter the inadequacy of this hypothesis by providing and discussing concrete examples of a wide variety of living systems in which the sodium hypothesis does not hold.

In light of all the evidence discussed in this section, the conjecture that sodium ions are responsible for the generation of the action potential will have to be rejected. It follows directly that the Hodgkin-Huxley model, being it based on the estimates of Nernst equation for the equilibrium potential of sodium ions, lacks of experimental support thus cannot be considered suitable for physical interpreatations.

## 4.1 Sodium independence in non-squid systems

More than one hundred years ago, Overton found the presence of $Na^+$ ions in the bathing medium of muscle and nerve cells not to be a necessary requirement for their excitability (Overton 1902). Specifically, he observed that $Na^+$ could be entirely substituted with lithium without causing any appreciable reduction in the response to stimulation. Since the work of Overton, many studies appeared on the possibility to mantain excitability in the absence of external $Na^+$ ions. A wide variety of preparations have been used so far for such investigations, and it would be impossible to report them all in this context. We thus mention here below only some among the ones that appear to us most significative. It is worth keeping in mind that each of them is sufficient, alone, to invalidate the $Na^+$ hypothesis in the system where this was tested.

The first studies we want to cite are the ones that were made by Osterhout and colleagues on the algal plant Nitella during the '30s. Nitella is a long investigated system in electrophysiology for the capability of its macroscopic cells to develop action potentials. In fact, before the "discovery" of the squid giant axon by Young (Young 1936, 1938), this algae was the most popular system for single-cell studies of excitation. There is no objective reason to believe the mechanisms underlying the electrical activity of Nitella to be qualitatively different from the ones of animal cells (Cole and Curtis 1938, 1939). This clarified, what Osterhout, both working alone and with colleagues, did, was the following: he isolated and bathed Nitella cells in distilled water for several days until they lost their excitability; taking care that no injury had occurred, he then added to the solution various compounds in trying to restore proper functioning. He found in this way that as little as 1 mM $CaCl_2$ was sufficient to make the cell excitable again. Not only, also ammonia, ammonium ions, guanidine as well as a number of organic compounds were shown to be capable of the same effects (Osterhout and Hill 1933, Osterhout 1935, 1940). No trace of sodium was added during these studies. Osterhout reached the precious conclusion that "*we should expect irritability [...] to be restored by any substance which can put the surfaces into a condition similar to that found in normal cells in winter* [13] *and it seems possible that this might be done by a variety of substances*" (Osterhout 1935, p.

[13] Note that in winter Nitella cells are excitable while in summer they are not

994).

Another interesting finding was made by Fatt and Katz in crab muscle fibers (Fatt and Katz 1951-53): in the tentative to extend the previous work done with Hodgkin on the squid giant axon (Hodgkin and Katz 1949), Katz (and Fatt) entirely replaced sodium with choline in the new system. Here is what the two wrote:

"*The effect of the substitution of choline for sodium was unexpected and striking; in no case were muscle fibres rendered inexcitable; on the contrary, the action potential became significantly larger, and many fibres which had previously given small local responses now produced large propagated action potentials. [...] The observation that the action potential is retained and, indeed, intensified when the external sodium had been totally replaced by choline is so surprising that we could not help suspecting some error.*" (Fatt and Katz 1953, p 186-187)

All the suspects being ruled out in the same publication, the two authors further investigated excitability of crab muscle fibers in $Na^+$ -free solutions and found that, besides choline, several quaternary ammonium ions were able to substitute for sodium. The studies received confirmation in the years that followed; in particular, Fatt and Ginsborg showed that crayfish muscle fibers were able to generate action potentials also when $Na^+$ was replaced by strontium (or barium): "*The presence of Na or* Mg*, in addition to* Sr*, did not affect the action potential*" (Fatt and Ginsborg 1958, p 542).

Before Fatt and Katz examined the effects of substitution of $Na^+$ in crustaceans, in fact even before the $Na^+$ hypothesis was conceived, the influence of quaternary ammonium ions had been extensively investigated on frog nerves by Lorente de Nó. The seminal work of the Spanish neurophysiologist was published in a very detailed 231-pages supplement on the Journal of Cellular and Comparative Physiology in 1949 (Lorente de Nó 1949). It was shown there that the ability to conduct impulses by small myelinated and unmyelinated frog nerve fibers which had been previously rendered inexcitable in sodium-free solutions, could be restored by tetraetylammonium, as well as several other quaternary ammonium ions. Also large myelinated fibers, whose electrical activity could not be restored at the time of the studies by the same treatment, were later found to be capable to regain excitability in $Na^+$ -free solutions: guanidinium as well as five other different onium ions turned out to be suitable for the purpose (Larramendi et al. 1956, Lorente de Nó et al. 1957).

Always in frogs, Koketsu and coworkers obtained that the excitability of isolated muscle fibers as well as spinal ganglion cells could be mantained in complete absence of $Na^+$ (Koketzu et al. 1958a,b, 1959). Very interestingly, in hydrazinium-solutions the action potentials were found to be "practically indistinguishable" from the ones in normal $Na^+$ -rich solutions (see Figure 3a).

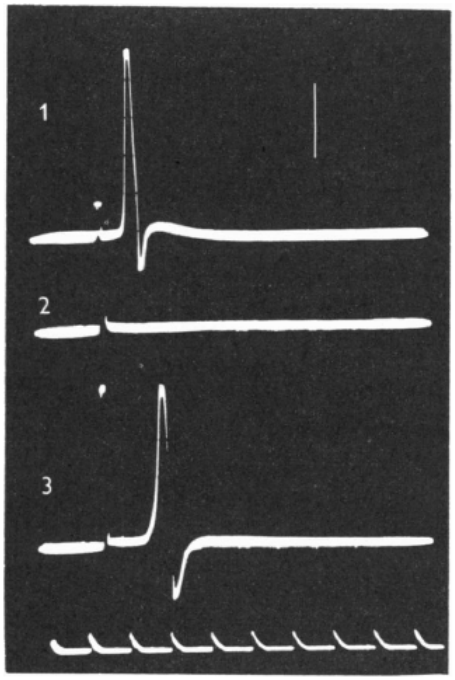


**Fig. 1**

Figure 3a: Action potentials from isolated frog's single muscle fibers in (1) $Na^+$-rich physiological solution; (2) 10 minutes after bathing in sucrose solution; (3) 5 minutes after immersion in $Na^+$-free hydrazine-containing solution.

It is worth citing directly a passage from the paper that appeared in Nature in 1958: "[...] *the striking similarity between 'hydrazinium spikes' and 'sodium spikes' suggests a single mechanism underlying the production of at least both brief responses, and apparently denies the exclusive necessity of external sodium for the generation of a 'normal' type of action potential in spinal ganglion cells. Moreover, it seems reasonable to assume that hydrazinium ion acts in the same*

*way as other onium ions, and it is not responsible for the actual transport of charge.*"

Not only Koketsu and colleagues found that frog neurons could still be artificially stimulated in the absence of external sodium, but that under the same conditions even synaptic transmission was perfectly normal (Koketsu and Nishi 1958c).

Now, it should be said that Hodgkin and Huxley were, at least after the publication of their model, well aware of most of the results just mentioned (see for example Hodgkin's book (Hodgkin 1964)). Despite of this, they both sustained in several occasions that the majority of excitable fibers conformed with the hypothesis that action potentials depend on an increase in $Na^+$ premeability. The limited relevance the two physiologists attributed to the overwhelming evidence discussed above in defence of the broad applicability of the results they themselves obtained working on the squid giant axon, is a position that can hardly be shared. To avoid misunderstandings, in confirmation of the general character of the studies cited, evidence is provided in the following that the squid axon does not to constitute an exceptionality, inasmuch as the $Na^+$ hypothesis does not hold for this system too.

## 4.2 Sodium independence in the squid giant axon

When talking about squid axons, one scientist comes immediately to the mind: Ichiji Tasaki. As one of the most bright and prolific neurophysiologists of the last century, Tasaki performed on squids works of primary importance for the understanding of the role of ions, in particular of sodium, in the process of nerve excitation.

It should be said that until the 60s there was no way to control the concentration of ions inside neurons, not even in the giant ones. So far, all the experiments that have been cited, included the ones performed by Hodgkin, Huxley, and Katz (see preceeding Chapters), had been performed ignoring which was

the effective ionic composition of the intracellular milieu. It was only in 1961 that Baker Hodgkin and Shaw on one side, Tasaki and colleagues on the other, independently managed to access and control the ionic environment inside the squid giant axon (Baker et al. 1961, Oikawa et al. 1961).

Already in 1963 Tasaki and Takenaka published the observation that the sensitivity of the amplitude of the action potential on the variations in the concentration of $Na^+$ ions inside neurons could not be explained in terms of Nernst's theory. In particular, they found that "*The overshoot was reduced by a large increase in the internal sodium, but the observed reduction was far smaller than is predicted by the Nernst equation applied to the* Na-*ion concentrations across the membrane*" (Tasaki and Takenaka 1963).

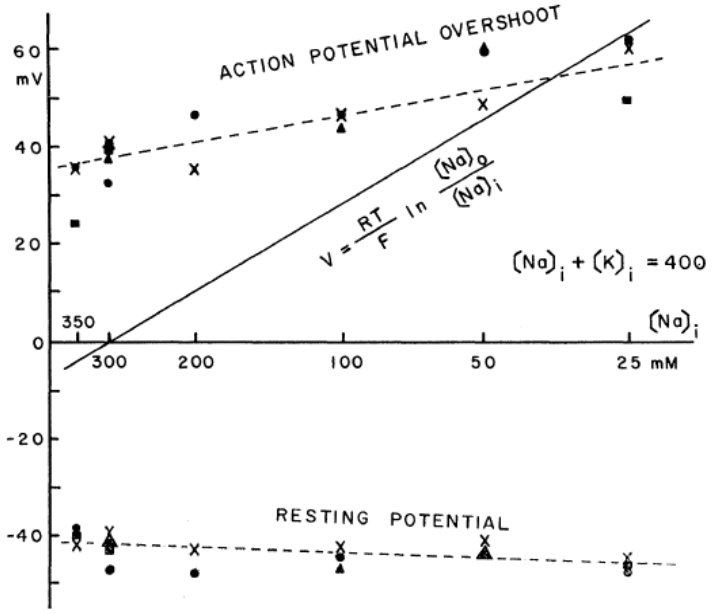


Figure 3b: Action potential overshoot at different concentrations of $Na^+$ inside the squid axon (dashed line on top). The continuous line represent the prediction derived from Nernst's theory. The lower part of the graph deals with the variation of the resting potential, but is not discussed in this thesis.

Figure 3b is reproduced from (Tasaki and Takenaka 1963). On the left hand side one can clearly see that the action potential overshoot significantly exceeds

the expectations deriving from Nernst's equation. Of special importance is that even in the absence of a gradient in the concentration of $Na^+$ ions across the membrane, i.e. when Nernst theory predicts no overshoot at all, a positive potential as high as 40 mV was recorded upon excitation. In 1964 the experments of Figure 3b were reproduced and confirmed (Tasaki and Luxoro 1964, Tasaki and Takenaka 1964).

While the findings on Nitella, crustaceans, frogs, and even mammals, could be ignored and labeled as special cases, the ones made on squids could not, as the latter were the original (and only) model systems where Hodgkin and Katz had directly tested their sodium hypothesis. Critics to the work of Tasaki and colleagues were published in 1965 by Hodgkin and Chandler in which the experimental techniques used by the Japanese physiologist were put into doubt. In particular, the "unexpected" results were claimed to be artifacts arising from the use of high-resistance electrodes as well as from their improper positioning inside the axon (Hodgkin and Chandler 1965a, b). Tasaki, Luxoro and Ruarte promptly reproduced the experiments following the technical modifications suggested by Hodgkin and colleague, and still obtained the same results (Tasaki et al. 1965). Not only, in the years that followed, Tasaki managed to elicit action potentials in squid giant axons under a wide variety of experimental conditions, including the complete absence of extracellular sodium and the presence of only salts of divalent cations such as for example $CaCl_2$ in the bathing medium (Tasaki 1982).

## 4.3 Evidence of sodium transmembrane flow

This chapter has been dedicated to the experimental evidence collected mainly between the 60s and the 70s against the validity of the sodium hypothesis. Given the way the latter hypothesis is presented in neuroscience and neuroscience-related textbooks (see in particular Kandel et al. 2000, and Purves et al. 2008), namely as an established fact, it would be natural to wonder wether there exists in fact a direct evidence, at least in some particular preparation,

that the transmembrane flow of sodium ions is responsible for the generation of action potentials. Regarding this issue, we want to remember here clearly that no such inopinable evidence has been provided so far, and that the sodium hypothesis remains, as in the mid 1900, an hypothesis in all excitable systems. In particular, of the experimental techniques which have been available up to nowadays, none allows to identify the transmembrane movement of any specific ion species ($Na^+$ included) while guaranteeing, at the same time, high temporal resolution. Even more specifically, none of the two techniques which are most often cited when dealing with the $Na^+$ hypothesis, namely radioactive tracers and voltage clamp, satisfy contemporarily the two necessary requirements just mentioned (Hodgkin 1951). Indeed, while the use of radioactive $Na^+$ guarantees ion specificitiy but is to slow to resolve millisecond single action potentials (see for example Keynes 1951), voltage clamp has a very high temporal resolution but does not allow to know the identity of the ions which cross the membrane during the voltage steps (Cole 1949).

Before closing this chapter, it seems worth to recall that the modern methods of visualisation of sodium movement by fluorescent chelators as well (Fleidervish et al. 2010, Baranauskas et al. 2013), cannot be considered appropriate for providing evidence of $Na^+$ - specific flow. As reported in the technical manual by one of the producing companies of such compounds (Invitrogen 2010), indeed, although these fluorescent $Na^+$ indicators are "*quite selective for* $Na^+$ *ions,* $K^+$ *has some effect on their affinity for* $Na^+$". Not only, the fluorescent signal "*is strongly affected by ionic strength and viscosity*" (Invitrogen 2010, section 21.1), both factors which are long known to undergo a sudden change during action potential propagation (Flaig 1947), thus unavoidably undermining the overall reliability of the results.

CHAPTER V

# Models of nerve excitation after 1952

As emphasized by the special issue that the Journal of Physiology dedicated last year to the achievements of Hodgkin and Huxley (see Introduction and citations therein), despite of having been conceived more than half a century ago, the model of the two Nobel-awarded physiologists is still nowadays the reference model for nerve excitation phenomena. This stated, it is worth remembering that other attempts to quantitatively describe action potentials appeared as well even after 1952; the purpose of the latter being most often that of proposing a set of equations which could have been simpler to handle from the analytical point of view, or that of including in a more comprehensive framework the phenomenological aspects which were left aside in the original picture by Hodgkin and Huxley.

Although it is beyond the purpose of this thesis to deal with the developement of theoretical modelling of nerve excitation after 1952, for the sake of completeness we decided to dedicate a short chapter to at least mention two of these tentatives: the FitzHugh-Nagumo and the Heimburg-Jackson models. For a detailed treatement of them, reference is made to the original publications (FitzHugh 1961, Heimburg and Jackson 2005).

## 5.1 The FitzHugh-Nagumo model

The Hodgkin-Huxley equations for the action potential represent a four-dimensional dynamical system which, despite of being capable of describing the voltage variations occurring during excitation across the membrane of a wide variety of biological preparations, is not easy to handle from the theoretical perspective. Already in 1961, FitzHugh proposed a simplification of the original model consisting of a two-dimensional system (FitzHugh 1961).

The reduction conceived by FitzHugh can be obtained from the Hodgkin-Huxley model on the basis of two observations (Murray 2002): first, the gating variables $n$ and $h$ have a much slower kinetics than $m$[14]; second, the model retains its characteristic features even if $h$ is set constant. Recalling the equations describing the dynamics of the gating variables in the Hodgkin-Huxley model:

$$\frac{dn}{dt} = \alpha_n(1-n) - \beta_n n$$

$$\frac{dm}{dt} = \alpha_m(1-m) - \beta_m m$$

$$\frac{dh}{dt} = \alpha_h(1-h) - \beta_h h,$$

the first one of these could be rewritten in the form

$$\tau \frac{dn}{dt} = n_\infty - n,$$

where $n_\infty = \frac{\alpha_n}{\alpha_n+\beta_n}$ is the value that $n$ eventually attains after a sufficient period of time, and $\tau = \frac{1}{\alpha_n+\beta_n}$ is the time-scale of the process; moreover, the variables $m$ and $h$ could be replaced in the Hodgkin-Huxley equation

$$I = C_M \frac{dV}{dt} + \bar{g}_K n^4 (V - E_K) + \bar{g}_{Na} m^3 h (V - E_{Na}) + \bar{g}_l (V - V_l) \qquad (41)$$

respectively by the limit value $m_\infty^3$, and by the constant $h_0$. In this way, the following two-dimensional system is obtained:

[14] For the meaning of $n$, $h$, and $m$, see Chapter III.

$$
\begin{aligned}
C\frac{dV}{dt} &= -\bar{g}_K n^4 (V - V_K) - g_{Na} m_\infty^3 h_0 (V - V_{Na}) - g_L (V - V_L) + I_{appl} \\
\tau \frac{dn}{dt} &= n_\infty - n
\end{aligned}
$$

Observing now that the $V$- nullcline and the $n$-nullcline of this system can be approximated respectively by a cubic function and a straight line (Murray 2002), one is led finally to the general form of the two dimensional system that takes the name of FitzHugh and Nagumo [15]:

$$
\frac{dv}{dt} = v(v-a)(1-v) - w + I \tag{42}
$$

$$
\frac{dw}{dt} = bv - \gamma w \tag{43}
$$

Here $v$ and $w$ are often related to, respectively, the membrane potential and a combination of the three gating variables of the Hodgkin Huxley model $n$, $m$, $h$; $a$, $b$, and $\gamma$ are positive constants.

Although abstract and not directly interpretable in biophysical terms, the system (42)-(43) was very successful especially among mathematicians due to its simplicity and at the same time capability to describe several of the salient features of action potentials.

[15] The year after FitzHugh published his model, Nagumo was able to build an electric circuit whose dynamics are described by the equations (42)-(43); for this reason, FitzHugh's model is commonly also known as the FitzHugh-Nagumo model.

## 5.2 The Heimburg-Jackson model

The Hodgkin-Huxley model accurately describes the electrical aspects of action potentials. It doesn't say anything, however, about the transient temperature and nerve volume variations which are known to be invariably present during excitation (Watanabe 1986). In 2005 Heimburg and Jackson tried to address these aspects as well by adopting a thermodynamical framework (Heimburg and Jackson 2005).

In Heimburg's and Jackson's perspective, action potentials are isoentropic density pulses (sound waves) spreading along neuronal lipid membranes. Restricting the problem to one dimension, the equation for the propagation of sound-waves in compressible media was thus adopted as the basis for the develoepement of the model. Specifically. in the absence of dispersion, this equation reads (Landau and Lifshitz 1987):

$$\frac{\partial^2}{\partial t^2}\triangle\rho^A = \frac{\partial}{\partial x}\left(c^2\frac{\partial}{\partial x}\triangle\rho^A\right), \tag{44}$$

where $t$ and $x$ are respectively the temporal and spatial variables; $\triangle\rho^A := \rho^A - \rho_0^A$ is a function of both $t$ and $x$ and represents the change in lateral density of the membrane, $\rho^A$ and $\rho_0^A$ being the instantaneous and equilibrium lateral densities; $c$ is the velocity of sound, which equals $1/\sqrt{\rho^A k_s^A}$ (Heimburg and Jackson 2005), $k_s^A$ being the isentropic lateral compressibility of the membrane.

From the experimental observation of frequency dependence of the velocity of sound in two-dimensional artificial membranes (Heimburg and Jackson 2005), i.e. dispersion, Heimburg and Jackson added to the right hand side of equation (44), the arbitrarily chosen dispersive term

$$-h\frac{\partial^4}{\partial x^4}\triangle\rho^A, \tag{45}$$

where $(h > 0)$ . Furthermore, given that close to the liquid-gel phase transition the isentropic lateral compressibility depends sensitively on the lateral density, displaying nonlinear properties (Heimburg and Jackson 2005), $c^2$ in equation (44) can be approximated by using its Taylor expansion in $\rho^A$ :

$$c^2 = \frac{1}{\rho^A k_s^A} = c_0^2 + p\triangle\rho^A + q(\triangle\rho^A)^2 + ... \tag{46}$$

where $p < 0$ and $q > 0$ are Taylor expansion coefficients which can be experimentally determined using artificial preparations such as pure lipid bilayers. Substitution of (45) and (46) into (44) leads eventually to the Heimburg-Jackson wave equation for nerve excitation:

$$\frac{\partial^2}{\partial t^2}\triangle\rho^A = \frac{\partial}{\partial x}\left[\left(c_0^2 + p\triangle\rho^A + q(\triangle\rho^A)^2\right)\frac{\partial}{\partial x}\triangle\rho^A\right] - h\frac{\partial^4}{\partial x^4}\triangle\rho^A. \qquad (47)$$

The authors showed that this equation admits solutions which qualitatively resemble nerve action potentials (solitary waves; see Appendix B).

By treating action potentials as density pulses spreading along the lipid membrane of neurons, the Heimburg-Jackson model implies, as unseparable phenomena related to nerve excitation, the presence of an electric pulse, a volume pulse (cellular swelling), and a temperature pulse (heat release and absorption). Lateral area density variations, i.e. changes in the packing of lipid molecules, imply indeed the alteration of the transbilayer electric field, the increase/decrease in the length of the hydrocarbon chains, and the exchange of heat with the surroundings (Heimburg and Jackson 2005). Interestingly, all these phenomena have been experimentally observed during action potential propagation (Cole and Curtis 1939, Tasaki 1989, Abbott and Hill 1958, Ritchie and Keynes 1985).

Before closing, it is important to note that, although accounting qualitatively for a variety of aspects of nerve excitation, the Heimburg-Jackson model does not provide a quantitative prediction for them. In particular, a thorough description of the action potential profile comparable to the one present in the Hodgkin-Huxley model is still missing. Further work is thus required to understand whether the framework adopted by Heimburg and Jackson will eventually be capable of providing a satisfactory physical explanation of nerve excitation.

CHAPTER VI

# Discussion

The mathematical model for the generation and propagation of nerve excitation proposed by Hodgkin and Huxley in 1952 has been critically analyzed. To this end, the scientific influences and roots of the two physiologists were first investigated so to neatly identify the theoretical assumptions underlying the hypotheses adopted as well as the motivation and reasoning that led to the developement of the quantitative aspects. The derivation of the differential equations constituting the model and the determination of the related parameters were then presented faithfully to the original works. A section dedicated to the critics of the model followed, in which the most fundamental assumption underlying the Hodgkin-Huxley model, namely the sodium hypothesis, was shown to be inconsistent on different levels: theoretical, experimental, and logical.

Despite of its inconsistencies, the sodium hypothesis has received broad acceptation in time up to the point that it constitutes nowadays one of the basic principles of our understanding of how neurons function. The reason why this could be defended by the vast majority of the scientific community in face of the experimental evidence is at least in part (if not mainly) due to the the attractive mathematical formalism used by Hodgkin and Huxley.

Among the quantitative models of nerve excitation that have been treated in the historical analysis in Chapter II, one can neatly discern between two different approaches: one physically grounded, starting from first principles and motivated by the analogy with known physical models (Nernst, Hill 1910, Lapicque), and one purely abstract based on the use of differential equations not at all related to any specific physiological process (Blair, Rashevsky, Hill 1936). The first approach allowed to speculate over the the physico-chemical processes involved in action potentials; the second had the significant advantage of precision

and flexibility deriving from the unconstrained origin of the mathematical formulation. Now, the Hodgkin-Huxley model is a mixture of the two approaches: although the authors adopted as a starting point the well-defined electric circuit analogue with Ohm's and Kirchhoff's laws, when it came to the description of the never-experimentally verified selective permeability changes to $Na^+$ and $K^+$ ions, fictitious equations with no physical nor physiological grounds were used. The abstract nature of the equations adopted was emphasized by Hodgkin and Huxley themselves:

"*The agreement* [of the model] *must not be taken as evidence that our equations are anything more than an empirical description of the time-course of the changes in permeability to sodium and potassium.* [...] *certain features of our equations were capable of a physical interpretation, but the success of the equations is no evidence in favour of the permeability change that we tentatively had in mind when formulating them*" (Hodgkin and Huxley 1952d)

It is worth spending few words on this passage, as it perfectly summarizes the error the two physiologists from Cambridge made when building their model. Hodgkin and Huxley declared the complete lack of an a priori physical ground for their equations, thus allowing their eventual substitution with any other sufficiently accurate mathematical description of the electrophysiological data. At the same time, however, they clearly state that whatever formalism one decides to adopt, this will in any case need to be "*a description of the time-course of the changes in permeability to sodium and potassium*". It is in this very fundamental basis that the error relies. The model originally conceived in 1952 and still nowadays so popular, describes with high precision something that, as shown in Chapter IV of the present thesis, does not conform with the experimental evidence. It is, thus, purely fictitious. Despite of this, the electric circuit analogue that the two physiologists used as a basis for their theory gave the illusion of the existence of a physical ground for it.

It is an unfortunate consequence of the high flexibility of the mathematical model Hodgkin and Huxley conceived to describe the process of nerve excitation, that unjustified assumptions were accepted without the least criticism. It is then far from the truth that "*the Hodgkin-Huxley model revealed mechanisms long before they could directly be observed*" (see Introductory Chapter); rather, the two physiologists from Cambridge provided a theoretical framework whose descriptive power is so strong that the disillusioned, objective interpretation of the experimental observations was, from then on, highly impared (note

that for any particular electrophysiological recording one can claim the presence of specific ion-channels with whatever invented dynamics). In such a difficult situation, only direct, crucial experiments could clarify the (in)validity of the underying assumption. It has been shown in this thesis that these experiments have been performed, and that they leave no possibility of interpretation.

It happened more than once that discussing with some colleagues at the Max Planck Institute in Goettingen, the claim was advanced that the Hodgkin-Huxley model, despite of the manifest inconsistencies with the experimental evidence as well as the incapability to account for certain not at all negligible phenomena that characterize nerve excitation, should nevertheless be used as a ground basis from which to start to develop a more accurate theory. It was foreseen that only a model that incorporates the Hodgkin-Huxley equations for the description of the electrical aspects of nerve excitation could ever provide a proper theoretical framework to interpret the phenomenon of action potentials. With respect to this issue, the present thesis is eloquent: by no mean the theory developed by Hodgkin and Huxley can be extended nor integrated to obtain a model that does not contradict the laws that govern nerve excitation.

Given that the Hodgkin-Huxley model has been ruled out, it becomes natural to wonder which could be then an interpretation of action potentials consistent with experimental evidence. Still, the work towards an alternative model for nerve excitation is ongoing (since recently also here at the MPI-Goettingen), but certain important considerations can already be made. In order to build a new (possibly valid) theory, one should identify which are the assumptions that we nowadays adopt that derive either directly or indirectly from the non-valid sodium hypothesis - among these there is certainly the hypothesis of the existence of ion-specific channels-. Only once the inconsistencies of our current common beliefs are identified, a model free from unjustified assumptions can be finally constructed. With respect to this, it seemed to us due to mention that here exist already models of nerve excitation that do not rely on the transmembrane flow of specific ions to explain the membrane depolarization (Heimburg and Jackson 2005; see Chapter V). For this purpose some models use, for example, piezoelectric effects occurring during the propagation of the action potential. Experimental observations showed that these phenomena in fact occur during excitation. It seems likely that such more physically-based approaches will be capable to provide a much clearer understanding of the phenomenon of nerve excitation than the one we have nowadays.

APPENDIX A

# Equivalence of Rashevsky's and Hill's theories

Rashevsky's model is given by

$$\frac{de}{dt} = KI - k(e - e_0)$$

$$\frac{di}{dt} = MI - m(i - i_0)$$

where I is the current, K, k, M and m are constants (with $m \ll k$), e and i are respectively the excitatory and the inhibitory factors. Excitation is assumed to happen when $e \geqq i$.

Hill's model is given by

$$\frac{dV}{dt} = bI - (V - V_0)/k'$$

$$\frac{dU}{dt} = \beta(V - V_0) - (U - U_0)/\lambda$$

where V is the excitatory process, U the threshold, b, k', β and λ are constants (with $\lambda \gg k'$). Excitation is assumed to happen when $V \geqq U$.

Starting from this last model, we have that

$$V = V_0 + be^{-t/k} \int_{\theta=0}^{\theta=t} Ie^{\theta/k'} d\theta \tag{48}$$

$$U = U_0 + \beta e^{-t/\lambda} \int_{\theta=0}^{\theta=t} (V_\theta - V_0) e^{\theta/\lambda} d\theta. \tag{49}$$

Substitution of (48) in (49) gives

$$U = U_0 + \beta b \frac{k'\lambda}{k' - \lambda} \left[ e^{-t/k'} \int_{\theta=0}^{\theta=t} Ie^{\theta/k'} d\theta - e^{-t/\lambda} \int_{\theta=0}^{\theta=t} Ie^{\theta/\lambda} d\theta \right].$$

Since excitation occurs once $U = V$, then we have that this condition translates into

$$\frac{\lambda - k'}{\beta\lambda k' + 1} e^{-t/k'} \int_{\theta=0}^{\theta=t} I e^{\theta/k'} d\theta = (U_0 - V_0)(\lambda - k')/b\beta\lambda k' + e^{-t/\lambda} \int_{\theta=0}^{\theta=t} I e^{\theta/\lambda} d\theta. \tag{50}$$

From Rashevsky's model we have

$$e = e_0 + K e^{-kt} \int_{\theta=0}^{\theta=t} I e^{k\theta} d\theta$$

$$i = i_0 + M e^{-mt} \int_{\theta=0}^{\theta=t} I e^{m\theta} d\theta$$

For the nerve to be excited, it is required that $e = i$ , thus that

$$e_0 + K e^{-kt} \int_{\theta=0}^{\theta=t} I e^{k\theta} d\theta = i_0 + M e^{-mt} \int_{\theta=0}^{\theta=t} I e^{m\theta} d\theta$$

which leads to

$$\frac{K}{M} e^{-kt} \int_{\theta=0}^{\theta=t} I e^{k\theta} d\theta = \frac{i_0 - e_0}{M} + e^{-mt} \int_{\theta=0}^{\theta=t} I e^{m\theta} d\theta. \tag{51}$$

Now, equation (51) is the same as (50), provided that

$$\frac{K}{M} = \frac{(\lambda - k')}{\beta\lambda k' + 1} \qquad \frac{(i_0 - e_0)}{M} = \frac{(U_0 - V_0)(\lambda - k')}{b\beta\lambda k'}$$

and that $k = \frac{1}{k'}$ and $m = \frac{1}{\lambda}$.

It follows that, by a proper choice of the parameters, every experimental result that can be described by one model can also be described by the other. The two theories are thus formally equivalent.

APPENDIX B

# The Heimburg-Jackson model: analytical considerations

Let's consider the Heimburg-Jackson equation (47):

$$\frac{\partial^2}{\partial t^2}\triangle\rho^A = \frac{\partial}{\partial x}\left[\left(c_0^2 + p\triangle\rho^A + q(\triangle\rho^A)^2\right)\frac{\partial}{\partial x}\triangle\rho^A\right] - h\frac{\partial^4}{\partial x^4}\triangle\rho^A$$

and look for solutions propagating without distortion, i.e. of the form $\triangle\rho^A(z)$ with $z = x - vt$. This allows us to write the equation above in the following form:

$$v^2\frac{d^2}{dz^2}\triangle\rho^A = \frac{d}{dz}\left[\left(c_0^2 + p\triangle\rho^A + q(\triangle\rho^A)^2\right)\frac{d}{dz}\triangle\rho^A\right] - h\frac{d^4}{dz^4}\triangle\rho^A. \tag{52}$$

Moreover, let's impose the condition that the solution has to be a solitonic wave $\triangle\rho^A(z) > 0$ for all $z \in \mathbb{R}$, and for which in particular $\lim_{|z|\to\infty}\frac{\mathrm{d}^k}{\mathrm{d}z^k}\triangle\rho^A(z) = 0$ for $k = 0, 1, ..., 4$, with $\triangle\rho^A(z)$ exponentially decaying. We can then integrate twice over the interval $(-\infty, z_0]$ and get, after rearrangement:

$$h\frac{d^2}{dz^2}\triangle\rho^A = (c_0^2 - v^2)\triangle\rho^A + \frac{1}{2}p(\triangle\rho^A)^2 + \frac{1}{3}q(\triangle\rho^A)^3. \tag{53}$$

Note now that for $|z|$ sufficiently large, $(\Delta\rho)^2$ and $(\Delta\rho)^3$ are very small compared to $(\Delta\rho)$. This means in turn that under such conditions the equation above is approximated by

$$h\frac{d^2}{dz^2}\triangle\rho^A = (c_0^2 - v^2)\triangle\rho^A, \tag{54}$$

whose solution reads

$$\triangle\rho^A = C_1 e^{z\sqrt{\frac{(c_0^2-\nu^2)}{h}}} + C_2 e^{-z\sqrt{\frac{(c_0^2-\nu^2)}{h}}}$$

with $C_1$ and $C_2$ constants. Here, three cases are possible depending on the sign of $\frac{(c_0^2-\nu^2)}{h}$:
(i) if $\frac{(c_0^2-\nu^2)}{h} < 0$, the solution is oscillatory, thus not solitonic and has to be excluded
(ii) if $\frac{(c_0^2-\nu^2)}{h} > 0$, from the condition imposed before ($\lim_{|z|\to\infty}\triangle\rho^A(z) = 0$) we have that $C_1 = 0$ for $z \to +\infty$ and $C_2 = 0$ for $z \to -\infty$. We observe that for $|z| \to \infty$, the solution goes as $\triangle\rho^A = Ce^{-|z|\sqrt{\frac{(c_0^2-\nu^2)}{h}}}$ with $C$ constant.

(iii) if $\frac{(c_0^2-\nu^2)}{h}=0$, the approximate equation is actually not anymore (54), but

$$h\frac{d^2}{dz^2}\triangle\rho^A=\frac{1}{2}p(\triangle\rho^A)^2.$$

By multiplying left and right hand sides by $\frac{d\triangle\rho^A(z)}{dz}$ and integrating, we arrive in this case, by separation of variables, to solutions of the form $u(z)=[az+b]^{-2}$. These are not however exponentially decaying, and will be neglected in the following.

Of the three cases, thus, only (ii) can be considered. It follows in this way from the analysis of the behaviour of the solution for $|z|$ sufficiently large, that the condition $|v|<c$ will have to be satisfied. It is observed furthermore that the solution has to be symmetric with respect to $z=0$.
In order to further proceed with the analysis, multiply both sides of eq. (53) by the derivative $\frac{d\triangle\rho^A(z)}{dz}$ and integrate. Observing that

$$2\frac{d\triangle\rho^A(z)}{dz}\frac{d^2\triangle\rho^A(z)}{dz^2}=\left(\frac{d\triangle\rho^A(z)}{dz}\right)^2,$$

we obtain the equation

$$h\left(\frac{d\triangle\rho^A(z)}{dz}\right)^2=(c_0^2-v^2)(\triangle\rho^A)^2+\frac{1}{3}p(\triangle\rho^A)^3+\frac{1}{6}q(\triangle\rho^A)^4. \qquad (55)$$

This latter allows to reason on the properties of the first derivative of $\triangle\rho^A(z)$. Referring to (55), the requirement of reality of the solution imposes $\frac{p^2}{9}-\frac{2}{3}q(c_0^2-v^2)\geq 0$, from which it follows that $v^2\geq\left(c_0^2-\frac{p^2}{6q}\right)$, implying in turn $\sqrt{c_0^2-\frac{p^2}{6q}}\leq|v|<c$ (combine with the condition found above from eq (54)). The sign equality between right and left hand sides moreover, requires

$$(\triangle\rho^A)^2\left[(c_0^2-v^2)+\frac{1}{3}p(\triangle\rho^A)+\frac{1}{6}q(\triangle\rho^A)^2\right]\geq 0.$$

Here, the stationary points of the solitonic solution are those which satisfy equality. Specifically:
(i) $z^*$ such that $\triangle\rho^A(z^*)=0$;
(ii) $z^*$ such that $(c_0^2-v^2)+\frac{1}{3}p(\triangle\rho^A(z^*))+\frac{1}{6}q(\triangle\rho^A(z^*))^2=0$;
If we look for maxima, we further require that the second derivative in $z^*$ is such that $\frac{d^2}{dz^2}\triangle\rho^A(z^*)<0$, which means, from eq. (53), that

$$(c_0^2-v^2)\triangle\rho^A(z^*)+\frac{1}{2}p(\triangle\rho^A(z^*))^2+\frac{1}{3}q(\triangle\rho^A(z^*))^3<0.$$

We can thus exclude case (i); case (ii) instead, leads to[16]

$$\triangle\rho^A(z^*) = -\frac{p}{q} \pm \frac{3}{q}\sqrt{\frac{p^2}{9} - \frac{2}{3}q(c_0^2 - v^2)}$$

together with the condition

$$(c_0^2 - v^2) + \frac{1}{2}p(\triangle\rho^A(z^*)) + \frac{1}{3}q(\triangle\rho^A(z^*))^2 < 0.$$

Observing that the latter can be obtained by adding the term $\frac{1}{6}p\triangle\rho^A(z^*) + \frac{1}{6}q(\triangle\rho^A(z^*))^2$ to eq (53) (divided by $\triangle\rho^A(z^*)$), which equals zero when evaluated in $z^*$, we have that this is satisfied if and only if

$$\frac{1}{6}p\triangle\rho^A(z^*) + \frac{1}{6}q(\triangle\rho^A(z^*))^2 < 0,$$

i.e. when, excluding $\triangle\rho^A(z^*) < 0$,

$$\triangle\rho^A(z^*) < -\frac{p}{q}.$$

Given that $q > 0$ from experimental observation, the inequality can be satisfied only if $p < 0$. This has been found to be the case from studies on artificial lipid bilayers (Heimburg and Jackson 2005). Under this condition, it follows that there can be only one maximum, which will correspond to $z^* = 0$ due to symmetry requirements. This maximum $\triangle\rho^A(z^*)$ is

$$\triangle\rho^A(z^*) = -\frac{p}{q} - \frac{3}{q}\sqrt{\frac{p^2}{9} - \frac{2}{3}q(c_0^2 - v^2)}.$$

This is the height of the soliton. We reach in this way the important conclusion that velocity is inversely related to height. In particular, from the Taylor expansion of the equation above for $v \to c_0$

$$\triangle\rho^A = 0 + \frac{3}{|p|}(c_0^2 - v^2) + \frac{9q}{|p|}(c_0^2 - v^2)^2 + ...$$

it is observed that for fast propagating waves $\triangle\rho^A$ becomes smaller and smaller. On the other hand, when the velocity reaches its lower limit $\left(c_0^2 - \frac{p^2}{6q}\right)$, we have that height reaches its maximum$-\frac{p}{q}$.

[16]Note that in the second equation $\triangle\rho^A(z*)$ has been simplified because we know that $\triangle\rho^A(z*) > 0$ .

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