INTRODUCTION TO

Electrophysiological Methods and Instrumentation

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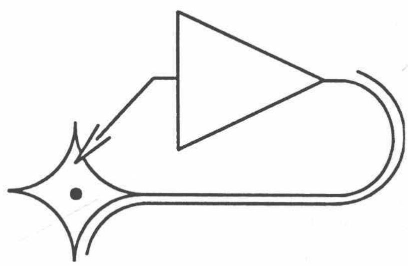

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To our teacher and colleague, Dr Robert C. Peters

Preface

Broadly defined, electrophysiology is the science and technique of studying the electrical phenomena that play a role in the life of plants and animals. These phenomena include the membrane potential, being ubiquitous among living cells, and its changes, which constitute signals playing an important part in the physiology of any organism. These signals may be slow changes caused by the changing concentration of some chemical substance, or the fast transient peaks called "action potentials" or "spikes", which arise by the fast opening of molecular "gates" in the membrane of neurons and similar types of electrically active cells.

Electrophysiological phenomena are the fastest signals in living nature: it has been found that, in directional hearing for example, time differences of less than 20 μs (between the arrival of sound in the left and the right ear) play a part. In addition to fast signalling, electrical processes have been proved to be useful for the sensitive detection of weak signals from the environment. Fish that are bestowed with electroreceptors—sense organs for electricity—have been shown to react to voltages as small as 1 μV across their body wall. The neural code, on the other extreme, is a strong, "sturdy" coding signal that will not be lost in long cables (nerves). By this virtue, the giraffe's brain can feel a mosquito crawling on its toes as precisely as one on its head. Equally powerful are the so-called motoric processes. Best known are the actions of our muscles, which are controlled and amplified largely by electrochemical processes. In addition, some electric fish emit strong pulses of electricity, over 500 V of tension, enough to stun their prey animals and to discourage most enemies. And, although plant life is usually more serene, electrical processes play an important part in metabolism; photosynthesis in particular. Some plants even generate "spikes", such as the well-known *Mimosa pudica* (sensitive plant). The fast withdrawal reflexes are mediated by electrically spreading action. Even though the chemical processes that support life as a whole may be important, studying the fast electrical processes is a fascinating branch of the life sciences, both for the basic satisfaction of our curiosity and for medical purposes.

To the general public, electrophysiological methods are best known from the latter category: ECG (electrocardiogram) and EEG (electroencephalogram) are terms well known from newspapers and television programmes. Apart from these famous clinical applications, a host of methods is in use, such as the recording from muscles (EMG or electromyogram) and from the eye (ERG, or electroretinogram), the electrical measurement of eye movements (electronystagmogram), the recording of nerve activity, the recording of the activity of single cells, whether nerve cells or not, and, through the "patch-clamp" technique introduced mainly by the Nobel laureates Neher and Sakmann, the recording of the activity of single ion channels, those tiny electrical gates in the membranes of all kinds of cells.

Working in electrophysiology implies, apart from a thorough knowledge of these phenomena, an equally well-founded control of one's equipment. In the early days of electrophysiological recording, amplifiers and other tools were often built by the physiologists themselves. Nowadays, many types of instruments for recording, processing and stimulation, versatile and almost perfect, can be delivered off the shelf from a host of reliable companies. *This does not, however, absolve the user from the obligation to maintain and use the apparatus properly, especially since a lack of knowledge about one's tools may lead to the publishing of erroneous results, which is a waste of time, money and intellectual effort.*

Therefore, despite the streamlined technology, the many electronic devices and computer algorithms available for filtering or post-processing of the signals and for the presentation of the data, all students of electrophysiology must gain proper insight into the working principles of their principal tools, and more specifically of vital components like preamplifiers and electrodes, which are connected to the preparation, the part of living nature that is to be studied. In planning experiments, with the concomitant purchase of instruments, one has to know the possibilities to choose from, and the consequences for the validity of the measurements.

Since most of these instruments depend heavily on electronic circuitry, introductory electronics takes the major part of this book (Chapter 2). In addition, however, we will spend some time on electrochemical processes, such as the ones that are inherent in the use of electrodes, salt bridges and the like, and on the electric—in fact also electrochemical—processes of life itself (Chapter 3). Although this is not meant as a book on electricity theory, we will inevitably spend a few pages on the most basic electrical quantities and processes such as charge, voltage and flow of current through so-called "passive" parts like resistors and capacitors, circuits like voltage sources and filters, and so on (Chapter 1). The complexity of electrophysiological signals and the knowledge to be derived from them lead to many forms of signal and data processing. The spectrum of methods (Chapter 4) may range from the simple recording of an ECG, judged by the eye, to the statistical processing of single-channel opening times by dedicated software and from a dot display of spikes on a computer screen to the analysis of stochastical point processes. In all cases, a proper introduction to the mathematical and electronic procedures involved leads to a better understanding of what is going on, and so reduces the risk of failure.

This book is intended for all students of electrophysiology, especially for readers without a formal training in electronics, signal analysis or electrochemistry, and hopes to serve as a thorough, yet easy-to-digest introduction that should lead all the way up from a first recognition of principles, to both understanding, and the routine application of, the various methods. To this end, this book uses informal language with qualitative explanations, yet using sufficient math to enable the reader to grasp the processes at a sufficiently quantitative level. Most of the jargon, essential if one is to discuss fluently in the area, will be introduced properly, while an index to the key words permits cross-references. This book is as concise as to be useful as a direct study guide, yet may also be used as a work of reference.

Because the principles of electronics are described in an elementary, yet detailed way, and because the discussion is extended to deal with digital instruments, including computer algorithms and mathematics, we hope this book will be useful as a general introduction in instruments and methods, also to people outside the field of electrophysiology. Additional matter is treated in appendices, such as the issue of safety in electrophysiological set-ups and the use of CRT monitors. Thematic literature references and an extensive keyword index complete the book.

Although written down by two authors, this book is the product of years of experience and cooperation with many colleagues and students. In the first place, we would like to thank our teacher and colleague Dr R.C. Peters, who laid the foundation for this book, and encouraged one of us (FB) to extend, improve and publish it over the many years of our cooperation. We owe him many contributions and suggestions. Appendix D, on CRT screen technology, was suggested by Dr R.J.A. van Wezel and commented on by him and by Mr J. Duijnhouwer. Dr K. Britten kindly provided Fig. D-5. Dr P.F.M. Teunis gave valuable comment and kindly provided the statistical data pertaining to gamma distributions. Many more people provided valuable comment on the first draft, among them Dr A.C. Laan, Mr W.J.G. Loos, Mr R.J. Loots, Mr A.A.C. Schönhage, Mr R. van Weerden, Dr T. Sanderson and several anonymous referees. We also acknowledge the encouragement by Prof. Dr A.V. van den Berg and Prof. Dr W.A. van de Grind. We also acknowledge the smooth cooperation of Dr J. Menzel, Ms M. Twaig and other people at Elsevier. Finally, we would like to thank all our students for explicit or implicit contributions, and for their patience with the earlier versions of this book.

F. Bretschneider
J.R. de Weille
Utrecht, 15 September 2005

Contents

1
Electricity

ELECTRICAL QUANTITIES

Although most of the vast field of electricity theory is outside the scope of this book, we will certainly deal with the handful of quantities that play an important part in electrophysiology—for the understanding of instruments as well as electrochemical and neurophysiological processes.

Electric Charge, Current and Potential

The basic quantity is the electric charge, buried in the atomic nucleus as what we call a positive electric charge, and in the electrons surrounding it, which we call negative charge. The unit of electric charge (abbreviated Q) is the coulomb (abbreviated C), defined in (macroscopic) electric circuits in the eighteenth century.

The underlying fundamental constant, found much later (in 1909, by R. Millikan), is the elementary charge, the charge of one electron, which amounts to 1.6021×10^{-19} C. Since this "quantum of electricity" is so small, most electric phenomena we will describe may be considered as continuous rather than discrete quantities.

By the nature of atoms, most substances, and indeed most materials in daily life, are neutral. Obviously, this does not mean that they have no charges at all, but that (i) the number of positive charges equals the number of negative charges and (ii) the opposite charges are so close together that they are not noticeable on a macroscopic scale. This means that a number of substances can be "teased" to release electricity, e.g. by rubbing together. This was indeed the way electricity was discovered in antiquity, and it was examined more systematically from the eighteenth century on. Many science museums are the proud owners of the large static electricity generators invented by, among others van Marum and Wimshurst. These machines generated rather high voltages (around 50 000 V), but at very low current strengths (1 μA), and so were not of much practical use.

Nowadays, most sources of electric energy are electrodynamic, such as the generators in our power plants, as well as in cars and on bicycles. In addition, the electrochemical processes, found originally by Galvani and Volta, are employed in the arrays of galvanic cells we call batteries and accumulators. Both forms of sources deliver the electrical energy at lower voltages (say, 12 V), but allow far larger currents to be drawn (hundreds of amperes in the case of a car battery).

This brings us to the most important quantities to describe electrical phenomena: the unit of tension, the volt (V), and the unit of current, the ampere (A). Note that the correct spelling of the units is in lower case letters when spelled out, but abbreviated as a single capital. In Anglo-Saxon countries, tension is often called "voltage". Both units have practical values, i.e. it is perfectly normal to have circuits under a tension of one volt or carrying one ampere in the lab or even at home. The definitions are derived from other fundamental physical quantities:

Charge (Q): one coulomb is defined as the charge of $6.241\,460 \times 10^{18}$ electrons.

Current (I): one ampere is a current that transports one coulomb of charge per second.

An overview of electrical quantities, their units and symbols is given in Appendix A.

The origin of the definition of tension, or potential difference, is a bit more intricate. The electrical forces that act on charges (or charged objects) depend not only on the field strength, but also on the distance travelled. Thus, electrical potential (abbreviated as U) is defined in terms of the amount of energy, or work (abbreviation W), involved in the movement of the charge from a certain point in the electric field to infinity (where the electrical forces are zero). If one does not move to infinity but from one point in the field to another point, less energy is involved. This is called the potential difference between the two points. Where to choose the two points will be a matter of practical, quantitative discussion.

Electrophysiologists measure what they call membrane potential by sticking one electrode into a cell. Theoretically, then, the reference electrode should be placed at infinity, where the potential is defined to be zero. In practice, however, the potential difference between inside and just outside the cell is measured. For this purpose, the potential just outside the cell can be considered to be sufficiently close to zero. This is caused by the fact that the membrane has a resistance that is many orders of magnitude higher than that of the fluids inside and outside the cell.

Other circumstances, however, change this view radically: many electrophysiological quantities are recorded entirely outside the cells, such as electrocardiogram, electroencephalogram and a host of signals from nerves and muscles. In this case the potential outside the cell cannot be considered zero! Instead, the potential difference between two extracellular points constitutes the whole signal. Nevertheless, the potential difference across the cell membrane is called potential in the long tradition of electrophysiology. The unit of tension, or potential difference, is the volt.

Tension (U): one volt is the tension between two points that causes one joule (J) of work (W) to be involved in carrying one coulomb of charge from one point to the other.

We use "involved" because the energy is either necessary for or liberated by the movement, depending on the direction.

Resistance

The concept of resistance stems directly from these fundamental quantities: if a certain current flows through an object as a consequence of a tension applied to this object, it exhibits the phenomenon of resistance, which is defined as the ratio of voltage to current.

Resistance (R): one ohm (Ω) is one volt per ampere.

These relations are remembered better in the form of formulas:

$$I = Q/t \quad \text{or} \quad \text{ampere} = \text{coulomb/second:} \quad 1\,\text{A} = 1\,\text{C/s}$$
$$U = W/Q \quad \text{or} \quad \text{volt} = \text{joule/coulomb:} \quad 1\,\text{V} = 1\,\text{J/C}$$
$$R = U/I \quad \text{or} \quad \text{ohm} = \text{volt/ampere:} \quad 1\,\Omega = 1\,\text{V/A}$$

The latter law is known as Ohm's law, and is very familiar to all people that handle electrical processes. It is often seen in two other forms, depending on which is the unknown quantity:

$$U = IR \quad \text{and} \quad I = U/R$$

This means that, knowing any two quantities, Ohm's law yields the third one. This is used very frequently. In electrophysiology, for instance, one needs to calculate electrode resistances from the voltage that develops when feeding a constant current through the electrode, and membrane resistances from measured current values together with the clamping voltage, and so on.

Resistance is the property of an object, such as a micropipette or a cell membrane. Solids, such as copper, and fluids, such as water, also have resistance, but the value depends on the dimensions of the body or water column. The resistance per unit of matter is called "specific resistance" or "resistivity". The dimension is Ωm ("ohm metre"). In electrochemistry, where the small unit system (cgs system) is still used frequently, the unit of resistivity is Ωcm. As a guideline, freshwater has a resistivity of about $1\,\text{k}\Omega\,\text{cm}$ ($10\,\Omega\,\text{m}$), seawater about $25\,\Omega\text{cm}$ ($0.25\,\Omega\text{m}$). Obviously, metals are better conductors, i.e. they have far lower resistivity values: in the order of $10^{-5}\,\Omega\text{cm}$.

The dimension "ohm metre" may seem odd at first, but is easily explained since the resistance is proportional to the length of a water column, and inversely proportional to the cross-section, which is width $\times$ height, or the square of the diameter. So, it is actually a simplification of $\Omega\,\text{cm}^2/\text{cm}$.

Other, related quantities we have mentioned already are power and energy, or work. The quantity energy (symbol W) has a unit called joule (J). The related, often more interesting quantity of energy per unit of time is called "power" (symbol P), and has the unit watt (W). So, the performance of loudspeakers, car motors and stoves is expressed in W. The longer they are used, the more energy is spent (which must be paid), but power is the best characteristic. Electrical power depends on voltage, current and, through Ohm's law, on resistance:

$$P = UI; \quad \text{or} \quad P = I^2R; \quad \text{or} \quad P = U^2/R \quad (1\,\text{W} = 1\,\text{VA} \quad \text{or} \quad 1\,\text{W} = 1\,\text{V}^2/\Omega)$$

and so on. Work is simply power times time:

$$W = Pt \quad \text{or} \quad W = I^2Rt$$

and so on. The latter formula is known as Joule's law.

Capacitance

The quantity to be discussed next is capacitance. This is the ability to store electric charge associated with a voltage. Now what is meant by "store"? The phenomenon shows up, either wanted or not, when two conducting wires, or bodies in general, are brought close together. If one of the conductors carries a positive charge, and the other one a negative charge, a (relatively high) voltage exists between the two. When brought closely together, however, the electric fields influence each other, thereby partially neutralizing the effect (If there are equal positive and negative charges or if the charges have the same centre of gravity, the net result would be zero charge, or neutrality. This is why atoms in general are neutral). In other words, by bringing two conductors together, the voltage decreases. Therefore, the charge is partially "hidden" or stored. The shorter the distance, and the larger the surface area, the more charge can be stored.

Note that this works only if the two conductors are separated by a very good insulator, such as a vacuum or dry air. Otherwise, a current would neutralize the charges. Other good insulators are glass, most ceramics and plastics. Note also that charge storage is different from what we saw with resistors: a voltage exists across a resistor only as long as a current is flowing through it; the moment the current stops, the voltage will be zero. A capacitance behaves differently. This can be seen by comparing electric quantities with hydraulic ones. Capacitance is an analogue of a vat or water butt. The amount of water is the analogue of an electric charge, the flow of water is the analogue of an electric current, and the water level corresponds to an electric voltage. When water flows in a vat, the water level builds up slowly, depending on the total amount of water poured in. In a small vat, a certain water level is reached with a smaller amount of water than in a large vat. The larger vat is said to have a larger storage capacity.

In the same way, a capacitor is a vat for electric charge, and the word "capacitance" is derived directly from this analogy. The unit of capacitance (symbol C) is farad (symbol F, after Faraday).

Capacitance (C): one farad is the storage capacity that causes a tension of 1 V to arise by transferring one coulomb of charge.

$$\text{or} \quad C = Q/V$$

Check the following derived formulas:

$$C = It/V; \quad C = t/R; \quad \text{and} \quad Q = CV$$

The capacitance exhibited by two conductors depends on distance, and hence on the form of the objects. Wires, spheres and irregular shapes have part of the surface area closer, and part farther from the other conductor. For two parallel plates, the capacitance can be calculated easily:

$$C = \varepsilon_0 \varepsilon_r A/d$$

Here, A is the surface area ($l \times w$ for a rectangle, πr^2 for a circle), d is the distance between the plates, ε_0 is a constant called the "absolute permittivity" of free space, also absolute dielectric constant, and has a value of 8.854×10^{-12} F/m. Finally, ε_r is called the "relative permeability" or "relative dielectric constant", often dielectric constant for short, and is determined by the material between the plates. By definition, vacuum has a dielectric constant of unity, air has