Electrophysiological Methods in Biological Research

J. BUREŠ
M. PETRÁŇ
J. ZACHAR

Československá akademie věd

SEKCE BIOLOGICKO-LÉKAŘSKÁ

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MUDr. Jan Bureš, MUDr. Mojmír Petráň, MUDr. Jozef Zachar

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Nakladatelství
Československé akademie věd

PRAHA 1960

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Publishing House
of the Czechoslovak Academy of Sciences

PRAGUE 1960

Czechoslovak Academy of Sciences

Scientific Editors

MUDR. JOSEF HOLUBÁŘ and MUDR. JOSEF IPSER

Translated by

MUDR. PETR HAHN

Foreign language Editor

JOSEPH CORT, M.D., Ph.D.

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Preface

During the last twenty years the electrophysiological methods are used to an ever increasing extent in biological and medical research. They represent today the most perfect analytical research tool, rendering a dynamic picture of processes occurring in living matter from individual cells to mammalian brain. Nevertheless electrophysiology is still used insufficiently in many disciplines. Its broader application is hindered primarily by lack of qualified specialists. The need for electrophysiologists is large and their training difficult.

In the present book an attempt has been made to join up theoretical and technical informations concerning electrophysiology with practical instructions for performing fundamental electrophysiological experiments. The authors work in different fields of electrophysiology since 1950. They were not trained in any renowned laboratory and were therefore forced to solve various methodical problems committing many mistakes and overcoming many obstacles. Perhaps their experience may help scientists who themselves intend to commence electrophysiological research and for whom the following chapters are mainly intended.

The authors wish to use this opportunity to thank all their colleagues from the Institute of Physiology of the Czechoslovak Academy of Sciences in Prague and from the Institute of Experimental Medicine of the Slovak Academy of Sciences in Bratislava, who helpet them so much in preparing this book. The authors are much indebted for valuable advice and stimulating criticism of various parts of the manuscript to Dr M. Brazier, Dr H. Grundfest, Dr P. G. Kostyuk, Dr H. W. Magoun, Dr G. Moruzzi and Dr G. D. Smirnov. They are especially grateful to Dr J. Holubář and Dr J. Ipser, the scientific editors who carefully read the whole manuscript.

J. Bureš, M. Petráň, J. Zachar

Prague, April 6 1960

Introduction

Electrophysiology is concerned with electrical phenomena occurring in living matter and with the effect of electrical currents on living matter.

That part of electrophysiology which is concerned with the basis of bioelectrical phenomena might be termed general electrophysiology. The subject matter can be divided as follows:

1) A study of electrical states, e. g. polarity of tissues, cells, organisms etc.
2) A study of changes in voltage or current in these structures.
3) A study of the effect of electric current on living matter.
4) A study of the electrical characteristics of cells and tissues in general (Walter 1956).

Applied electrophysiology, on the other hand, makes use of certain electrical manifestations during activity of some system for analysing this system itself. The use of electrophysiological technique for solving specific problems concerning the function of excitable structures may serve as an example.

The object of such research, however, is not the problem of electrogenesis or the functional significance of electrical potentials, but that of analysing relationships between certain electrical phenomena and functions, between functional elements, between the stimulus and the response etc.

In such a case the action potential is only an expression of the nerve impulse, a fundamental unit of information by which various excitatory structures are mutually connected. Studying the movements of such signals along parts of the nervous system makes it possible to study its organisation, to clarify the interaction of individual elements, the formation and transmission of more complex information. The same is valid not only for action potentials, but also for more complex electrical phenomena (e. g. spontaneous EEG) which represents the sum of activity from extensive synaptic areas.

Used in such a way electrophysiology is nothing but a research tool (often the only one available), sometimes merely completing information obtained otherwise.

According to the system studied, or to other criteria, electrophysiology may be subdivided, e. g. into electrophysiology of plants and animals, skin, muscle, nervous system etc.

Historically the roots of electrophysiology are found in the controversy between Galvani and Volta concerning the interpretation of the experiment of Galvani (1791). Galvani explained his findings by the concept of animal electricity and Volta by postulating currents due to different metals connected by salt solutions.

This question could only be settled definitely when adequate measuring instruments were available. In 1819 Oerstad found that a magnetic needle is deflected under the influence of a galvanic current, and this led to the construction of galvanometers. In 1825 Nobili demonstrated the existence of muscle currents by means of a galvanometer of his own design. In 1845 Carlo Matteucci used a nerve-muscle preparation as a sensitive biological indicator of the presence of currents in another contracting muscle.

It is due to the book: "Untersuchungen über thierische Elektricität" by du Bois-Reymond that electrophysiology became an independent discipline. This author developed the technique of stimulation (inductorium) and recording using a galvanometer. He demonstrated two fundamental types of bioelectrical potentials — the resting and action potentials. Thus the 60-year-old controversy between Galvani and Volta was finally resolved.

Further knowledge concerning living matter, and especially nerves and muscles, increased with the development of electrophysiological techniques — particularly of recording apparatus. Sensitive mirror galvanometers and the telephone came into use in the 19th century (Wedensky 1883). In 1873 Lippman described the capillary electrometer, which permitted a more detailed study of electrical phenomena in living tissues.

At the beginning of the 20th century electrophysiology began to develop rapidly. Einthoven invented the string galvanometer and used it to record the ECG (1899). Electrophysiology thus moved from the laboratory into the wards and interest in this subject increased.

Further development of electrophysiology was due to the introduction of radiotechniques. The thermionic vacuum valve was invented, making it possible to amplify very small electrical voltages recorded from different tissues.

In 1922 the cathode-ray oscilloscope was introduced by Erlanger and Gasser. This marked the beginning of modern electrophysiology. Between the twenties and forties electrophysiology developed rapidly. Although the oscilloscope was already in use (it reached Europe in 1929 — Rijlant) the string galvanometer made possible the discovery of the electroencephalogram by Prawditz-Neminski (1925) and Berger (1929—1939). After Gibbs, Davis and Lennox (1935) discovered epileptic rhythms in the EEG and Walter (1936) applied this technique to localising tumours in the brain, electroencephalography soon became an independent applied discipline.

During that period electrophysiology was mainly applied to a study of the nervous system and soon became the dominant technique in this field. Muscular contractions as indicators of nervous activity were used less and less. Many physiologists were attracted by the possibility of studying an impulse wave from its origin in receptors to the central nervous system. They improved the methods used and made important discoveries.

Just before World War II, papers appeared indicating the beginning of a new phase in electrophysiology. Hodgkin and Huxley (1939), Cole and Curtis (1942) measured membrane potentials with longitudinal microelectrodes introduced into giant fibres. They found that the action potential measured with the microelectrode was larger than the membrane potential. At the same time the discovery of depolarisation at the myoneural junction (Schaefer and Haas 1939, Eccles and O'Connor 1939) opened up a new area of electrophysiology — synaptic potentials as a specific electrical manifestation of activity at the synapses. In 1940—1942 Schaefer systematically described electrophysiology.

After 1945 the latest phase of electrophysiology appeared. Microelectrode technique was enriched by transmembrane intracellular recording (Ling and Gerard 1949). The discovery of overshoot of the action potential over the membrane potential and thus the fall of Bernstein's hypothesis (1902), resulted in the formulation of a new ionic hypothesis (Hodgkin and Katz 1949; Hodgkin, Huxley and Katz 1952) and this in turn stimulated further development of intracellular recording techniques and other methods.

Using the most recent techniques, synapses, cells and nuclei deep in the central nervous system are studied not only in anaesthetised but also in normal animals.

Further development of electrophysiological methods will probably be determined by two factors:

1) The development of new electronic measuring and control apparatus used in other branches of science and industry which can be taken over directly by electrophysiology or adapted for its purposes.

2) The elaboration of new techniques of recording electrical potentials from living objects — particularly microphysiological methods and multilead recording.

Two characteristic trends can clearly be seen in contemporary electrophysiology: a) a more profound and exact analysis of individual data and b) an increase in the number of simultaneously obtained data. For practical purposes the two trends oppose each other to a certain extent and hence one or the other predominates in any given experimental work. In future, their further development will require the construction of special apparatus permitting quantitative treatment of the data obtained with the help of statistical theory.

It can be seen that electrophysiology has been primarily employed in the study of the nervous system, and this is true even today. It is most frequently used in research into the following problems:

1) The relationship between stimulus and response in sense organs.

2) The afferent course of peripheral signals, their connections in the CNS and reflex efflux.

3) Definition of functional-anatomic relationships between different structures of the CNS, by stimulation and extirpation with simultaneous recording.

4) Correlation of spontaneous and evoked electrical activity in different nerve structures with metabolism.

5) Correlation of spontaneous and evoked electrical activity in nerve structures with the animal's behaviour.

6) The study of the space-time dynamics of physiological and pathological nerve processes with clear cut electrophysiological manifestations (e. g. an epileptic seizure).

Concrete examples for the majority of these applications are given in chapters 3—9. The technique as well as the character of the data obtained are described.

The above, of course, does not exhaust by far the fields in which electrophysiological methods may be used. Its more extensive use today is not hindered so much by an isufficiency of suitable apparatus as by a lack of qualified investigators.

Great demands are made on a modern electrophysiologist. In addition to being acquainted with the physiology of the system studied, he must know the principles on which his instruments and apparatus work and the latter's capabilities and limitations. In the past, electrophysiologists themselves constructed and designed their own apparatus. Today this is no longer the case. Good electrophysiological laboratories have engineers on their staff or are supplied with suitable apparatus from factories. Yet the investigator must still know his equipment. This is all the more important if fundamental problems are being studied. In such cases the scientist must seek new approaches and must thus be well acquainted with electronics. The words of I. P. Pavlov are still not out of date in this connection: "Science progresses in steps depending upon the success of techniques. Each improvement in technique raises us a stage upward, from which a new horizon is uncovered containing phenomena not known before".

I

The theoretical basis of electrophysiological phenomena

A. Some fundamentals of electrochemistry

In order to understand the theories concerning the nature of bioelectrical potentials it is essential to have at least an elementary knowledge of electrochemistry. Only a short survey of the subject is given here, for detailed information the reader is asked to consult any textbook on physical chemistry (Mac Innes 1939, Höber 1945, Brdička 1952, Kiryeyev 1951).

Electrode potential

A potential difference arises between two different metal conductors immersed in a solution. The value of the potential depends upon the nature of the metals and the composition of the solution. Nernst (1889) attributed the existence of this potential to a tendency of the metals to discharge cations into the solution. This "dissolving pressure" is the greater, the less firmly electrons are bound to metal atoms, i. e. the less "noble" the metal. Metal cations of the solution, on the other hand, show a tendency to transmit their positive charge to the electrode. This is the stronger, the higher their concentration and thus their osmotic pressure. Both these processes result in the formation of an electric double layer at the phase boundary between the metal and the solution. The orientation of this double layer depends upon the mutual relations of "dissolving" and osmotic pressures.

If the former preponderates, the electrode is negative to the solution (the electrode loses positive charges to the solution); if the latter predominates, the electrode is positive (the electrode receives positive charges from the solution).

The theoretical value of the potential difference produced under such conditions at a single phase boundary is defined by the relation

$$V = V_0 + \frac{\mathrm{R}T}{n\mathrm{F}} \ln a \tag{1}$$

where V_0 is a constant for the given electrode, temperature and unit activity of cations.

R is the gas constant (8·314 Voltcoulomb . mol^{-1} . degree Kelvin^{-1})

T the absolute temperature ($t + 273°\mathrm{C}$)

n the number of elementary charges on one cation

F Faraday constant (96 500 Coulombs)

a the activity of cations in the solution (product of molar concentration c and activity coefficient f).

Since in dilute solutions the activity coefficient is nearly one, concentration (c) may replace activity (a) in equation (1).

After changing natural logarithms to Briggsian ones we obtain for V:

$$V = V_0 + 2{\cdot}303 \frac{\mathrm{R}T}{n\mathrm{F}} \log a \tag{2}$$

and for $n = 1$, $t = 20°\mathrm{C}$

$$V = V_0 + 0{\cdot}0582 \log a$$

or for $n = 1$, $t = 25°\mathrm{C}$

$$V = V_0 + 0{\cdot}0591 \log a$$

This potential difference cannot be measured directly, since at least two such phase boundaries must be present in a galvanic cell. The hydrogen electrode is used as standard reference in most cases (c. f. page 18).

If the electrode is such that quantitative, but not qualitative changes occur when a current is passing and if those changes are completely reversible on reversing the current, then it belongs to the type of reversible(non- polarisable) electrodes. These are:

1) Metal electrodes immersed in a solution containing cations of the same metal (e. g. Zn in $ZnSO_4$, Cu in $CuSO_4$ etc.).

2) Metal electrodes covered with a layer of a poorly soluble salt of this metal, in a solution containing the anion of that salt (e. g. Ag covered with a layer of AgCl or Hg covered with Hg_2Cl_2 in contact with a solution of some chloride. This latter kind of electrodes is often used in electrophysiology. A current passing in one direction carries ions from the electrode into the salt layer. If the current is reversed ions accumulate at the electrode. The system thus remains qualitatively unchanged and only small changes in ion concentration occur in the vicinity of the electrode. These do not have a significant effect on the e. m. f. of the cell.

Two electrodes immersed in a solution form a galvanic cell, characterised by potential differences produced between its individual parts.