

8203466

8203466

**Electrophysiological  
Methods  
in Biological  
Research**

J. BUREŠ  
M. PETRÁŇ  
J. ZACHAR

Československá akademie věd

SEKCE BIOLOGICKO-LÉKAŘSKÁ

ELECTROPHYSIOLOGICAL  
METHODS  
IN BIOLOGICAL  
RESEARCH

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

3735058

**ELEKTROFYSIOLOGICKÉ  
METODY  
V BIOLOGICKÉM  
VÝZKUMU**

Nakladatelství

Československé akademie věd

PRAHA 1960

UTP/ E701 MUDr. Jan Bureš, MUDr. Mojmír Petráň, MUDr. Jozef Zachar

外文书库

8202456

ELECTROPHYSIOLOGICAL  
METHODS  
IN BIOLOGICAL  
RESEARCH



Publishing House

of the Czechoslovak Academy of Sciences

PRAGUE 1960

353  
B. 95-2

**Czechoslovak Academy of Sciences**

*Scientific Editors*

**MUDR. JOSEF HOLUBÁŘ and MUDR. JOSEF IPSEK**

*Translated by*

**MUDR. PETR HAHN**

*Foreign language Editor*

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

© by Nakladatelství Československé akademie věd, Praha 1960

# Contents

Preface - . . . . .	9
Introduction - . . . . .	11
<b>CHAPTER I</b>	
<b>Theoretical basis of electrophysiological phenomena</b> (by J. Bureš) - . . . . .	15
A. Some fundamentals of electrochemistry - . . . . .	15
B. The membrane theory of bioelectric phenomena - . . . . .	23
<b>CHAPTER II</b>	
<b>Electrophysiological apparatus and technique</b> (by M. Petráň) - . . . . .	30
Introduction - . . . . .	30
Stimulation technique - . . . . .	31
Physical effect of the stimulus on the tissue - . . . . .	31
Physical characteristics of the stimulus - . . . . .	33
Main requirement for a stimulator - . . . . .	35
Kinds of stimuli - . . . . .	35
Harmonic voltage - . . . . .	35
Nonharmonic voltage - . . . . .	39
Voltage step. Rectangular pulses. Damped oscillations. Sawtooth oscillations. Repetitive stimuli	
Stimulators - . . . . .	45
Mechanical stimulators - . . . . .	45
Transformers - . . . . .	47
Electronic stimulators - . . . . .	50
Generators of harmonic oscillations - . . . . .	51
Generators of nonharmonic oscillations - . . . . .	55
Astable multivibrator. Monostable multivibrator. Bistable multivibrator. Synchronisation and triggering of multivibrators	
Blocking oscillator - . . . . .	66
Glow-discharge and thyatron oscillators - . . . . .	70
Pulse shaping circuits - . . . . .	76
Determination of the shape. Time relations. Multivibrators. Phantastron	
Limiters - . . . . .	81
Assembling a stimulator - . . . . .	86
Recording technique - . . . . .	91
The fundamental characteristics of signals and their sources - . . . . .	91
Recording apparatus - . . . . .	92
Amplifiers - . . . . .	94
Valves - . . . . .	94
Some interference phenomena in amplifiers - . . . . .	98

Noise. Microphony - . . . . .	98
Elements of A. C. voltage amplifiers - . . . . .	101
Coupling of stages. Frequency responses. Grid bias and cathode bias. Screen grid voltage. Types of A. C. voltage amplifiers	
Power amplifiers and impedance transformers - . . . . .	108
Electrometric amplifiers - . . . . .	108
Cathode follower - . . . . .	109
D. C. Amplifiers - . . . . .	116
Amplifying D. C. voltages with A. C. amplifiers - . . . . .	121
Recording devices - . . . . .	123
Moving coil, moving magnet and moving iron instruments. Capillary electrometer, string galvanometer, piezoelectric electrometer. Cathode-ray tube	126
Cathode-ray oscilloscope. Amplitude calibration and time marking - . . . . .	128
Mechanical and photographic recording - . . . . .	135
Simultaneous recording of electrical and non-electrical quantities - . . . . .	140
Location of faults and interferences - . . . . .	143
Faults - . . . . .	143
Interferences - . . . . .	147
Stimulation artifacts. Artifacts due to improper recording of bioelectric potentials. Determination of artifacts. Interferences limiting the recording of low amplitude signals	
Electrodes - . . . . .	158
Calomel and silver-silverchloride electrodes. Metal microelectrodes. Glass microelectrodes	

### CHAPTER III

<b>General electrophysiology of cells and tissues</b> (by J. Bureš) - . . . . .	166
A. Electric potentials of cells - . . . . .	166
Membrane potentials of large plant cells. Membrane potential of nerves. Membrane potential of a muscle fibre	
B. Electric potentials of tissues - . . . . .	175
Polarity of frog skin. Positive demarcation potential of gastric mucosa. Cell dipoles in series	
C. Electric phenomena in plants - . . . . .	186
Bioelectric potential of photosynthesis. Resting electric polarity of plants and the electric response of plant tissue to stimulation. Potential changes accompanying leaf movement in <i>Mimosa</i>	
D. Electric polarity in the animal organism - . . . . .	195
Electric phenomena of early embryogenesis in the chick	

### CHAPTER IV

<b>Electrophysiology of isolated excitable structures in vitro</b> (by J. Zachar) - . . . . .	199
A. Electrical manifestation of a nervous impulse - . . . . .	199
The action potential of peripheral nerves. The action potential of isolated nerve fibres	
B. Propagation of nervous impulses - . . . . .	214
Measurement of conduction velocity of a nervous impulse. Relation between	

	the fibre size and conduction velocity. Extrinsic potentials. Law of independent conduction. Interaction between nerve fibres	
C.	The initiation of nerve impulses - - - - - Electrotonus and nerve excitability (Change in nerve excitability near the cathode and anode. Werigo's cathodic depression and the phenomenon of Woronzov. Electrotonic potentials) Virtual cathodes. Strength-duration curve and chronaxie. Accomodation. The local response of a nerve. The effect of sodium ions on the production of an action potential	229
D.	Recovery processes following a nerve impulse - - - - - After-potentials. The absolute and relative refractory period. The super-normal and subnormal period. Maximal rhythm of a nerve fibre. The lability of Wedensky	245
E.	Electrophysiology of the isolated skeletal muscle - - - - - Action potential of a skeletal muscle. Biphasic and monophasic action potentials. The latent period of the mechanical response. Transmission on peripheral synaptic junctions - - - - -	252 255
F.	Neuromuscular transmission in skeletal muscle - - - - - Electrophysiological localisation of the end-plates in skeletal muscle. End-plate potentials. Repetitive stimulation of the end-plate. Excitation of the end-plate by acetylcholine	255
G.	Synaptic transmission in a sympathetic ganglion - - - - - Action potentials from the superior cervical ganglion. Synaptic potentials in the superior cervical ganglion. Occlusion and facilitation in the superior cervical ganglion. Recovery cycle in the sympathetic ganglion	267
 CHAPTER V		
	<b>Electrophysiology of peripheral excitable structures in situ</b> (by J. Zachar) - - - - - Recording of an impulse in a volume conductor. Impulse activity of somatic and vegetative nerves. Impulse activity of muscles in situ. Electromyogram	276
 CHAPTER VI		
	<b>Electrophysiology of receptors</b> (by J. Zachar) - - - - -	290
A.	Models of sensory organs - - - - - The effect of D. C. current on the isolated nerve fibre	290
B.	Impulse activity in sensory nerve fibres - - - - - Afferent impulses from muscles. Stretch responses	293
C.	Receptor potentials - - - - - The receptor potential of a muscle spindle. The electroretinogram. The electroolfactogram	297
 CHAPTER VII		
	<b>Electrophysiology of the spinal cord</b> (by J. Zachar) - - - - - Electrophysiological manifestations of a monosynaptic and polysynaptic reflex arc. Facilitation and inhibition in the motoneurone. Relation between afferent influx and efferent efflux in the spinal monosynaptic arc. The recovery cycle in the motoneurone. Synaptic potentials in a monosynaptic reflex arc. Post-tetanic potentiation	306



CHAPTER VIII

<b>Electrophysiology of the cerebral cortex</b> (by J. Bureš) . . . . .	320
A. Electroencephalography and electrocorticography in general . . . . .	320
B. Spontaneous EEG and ECoG in animals . . . . .	325
C. Spontaneous EEG during anesthesia . . . . .	330
D. Cortical primary responses . . . . .	335
Localization of primary responses in rat and cat. Primary responses to repeated stimuli. Secondary response	
E. Responses of the cerebral cortex to direct electrical stimulation . . . . .	350
F. Mapping of nervous pathways in the central nervous system using neuro-nography . . . . .	355
G. Depth recording in the cerebral cortex . . . . .	362
H. Antidromic and orthodromic stimulation of pyramidal paths . . . . .	366
I. Steady potentials and impedance of the cerebral cortex . . . . .	371
J. Spreading depression . . . . .	376
K. Theoretical basis of recording electric potentials in a volume conductor . . . . .	386

CHAPTER IX

<b>Electrophysiology of subcortical structures</b> (by J. Bureš) . . . . .	392
A. The stereotaxic method . . . . .	392
B. Primary responses in the subcortical centres of afferent systems . . . . .	399
C. Electric activity of the cerebellum . . . . .	404
D. Nonspecific subcortical influences on the cerebral cortex . . . . .	409
E. Electrical activity of the hippocampus . . . . .	414
F. Electrophysiological signs of an epileptic seizure . . . . .	419
<b>Appendix I</b> Stereotaxic atlases for the cat, rabbit and rat (by E. Fiková and J. Maršala, Institute of Anatomy, Charles' University, Prague) . . . . .	426
References . . . . .	468
Author index . . . . .	493
Subject index . . . . .	501

## 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 helped 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 Elektrizität" 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 19<sup>th</sup> 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 20<sup>th</sup> 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 insufficiency 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".

# 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





$$V = V_0 + \frac{RT}{nF} \ln a \quad (1)$$

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<sup>-1</sup> . degree Kelvin<sup>-1</sup>)

$T$  the absolute temperature ( $t + 273^\circ\text{C}$ )

$n$  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  $V$ :

$$V = V_0 + 2.303 \frac{RT}{nF} \log a \quad (2)$$

and for  $n = 1$ ,  $t = 20^\circ\text{C}$

$$V = V_0 + 0.0582 \log a$$

or for  $n = 1$ ,  $t = 25^\circ\text{C}$

$$V = V_0 + 0.0591 \log a$$

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<sub>4</sub>, Cu in CuSO<sub>4</sub> 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<sub>2</sub>Cl<sub>2</sub> 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.